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MICROBIAL EXOENZYME ACTIVITY AT THE HERON ISLAND OCEAN DUMPSITE (BAY OF CHALEUR): 10 YEARS AFTER THE DISPOSAL OF DREDGED SEDIMENT FROM DALHOUSIE, NEW BRUNSWICK
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K. Lee, K.L. Tay, E.M. Levy, C.N. Ewing and S.E. Cobanli

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MICROBIAL EXOENZYME ACTIVITY AT THE HERON ISLAND OCEAN DUMPSITE (BAY OF CHALEUR): 10 YEARS AFTER THE DISPOSAL OF DREDGED SEDIMENT FROM DALHOUSIE, NEW BRUNSWICK

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ABSTRACT

Lee, K., K.L. Tay, E.M. Levy, C.N. Ewing and S.E. Cobanli. 1990. Microbial exoenzyme activity at the Heron Island ocean dumpsite (Bay of Chaleur): 10 years after the disposal of dredged sediment from Dalhousie, New Brunswick. Environment Canada, Ocean Dumping Report 5: iii + 82 p.

Microbial exoenzyme activity in sediments from the Heron Island ocean dumpsite, Bay of Chaleur, New Brunswick, was measured with fluorogenic substrates. Elevated rates of cellulase activity at the dumpsite center were positively correlated with Cd, Pb and Zn concentrations in the sediment. In contrast, protease and phosphatase activity in the vicinity of the dumpsite was low and positively correlated with the vector distance from the dumpsite.

The effect of Dalhousie Harbour sediments on bacterial exoenzyme activity in sediments, collected from an uncontaminated "control" site in the Bay of Chaleur, varied with the source of the harbour sediment and enzyme assay used. While protease activity in "control" sediment was suppressed by the addition of contaminated harbour sediments, cellulase and phosphatase activity was stimulated in some instances. Experimental results support the use of multiple assay systems for assessment of pollutant impact on the marine environment.

RÉSUMÉ

Lee, K., K.L. Tay, E.M. Levy, C.N. Ewing et S.E. Cobanli, 1990. Activité d'exoenzymes microbiens au site d'immersion de déchets de l'île aux Hérons (Baie des Chaleurs): 10 ans après l'immersion de sédiments dragués de Dalhousie, Nouveau-Brunswick. Immersion de déchets en mer, rapport 5, Environnement Canada: III + 82 p.

L'activité d'exoenzymes microbiens dans les sédiments du site d'immersion de l'île aux Hérons, Baie des Chaleurs, Nouveau-Brunswick, fut mesurée à l'aide des substrats fluorogéniques. Des taux élevés d'activité cellulase au centre du site d'immersion démontraient une corrélation positive avec les concentrations de Cd, Pb, et Zn dans le sédiment. Par contraste, l'activité protéase et phosphatase alentour du site d'immersion était faible et démontrait une corrélation positive entre la distance vectorielle et le site d'immersion.

L'effet des sédiments du port de Dalhousie sur l'activité d'exoenzymes bactériens aux sédiments prélevés d'un site témoin non-contaminé, dans la Baie des Chaleurs, était variable même selon la source du sédiment portuaire et le test d'enzyme utilisé. Alors que l'activité protéase dans le sédiment témoin était étouffé par l'ajout de sédiments portuaires contaminés, l'activité cellulase et phosphatase était stimulée en certains cas. Les résultats expérimentaux appuient l'utilisation de systèmes multi-tests pour l'évaluation de l'impact de substances polluantes sur l'environnement marin.

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1.0 INTRODUCTION

Dalhousie Harbour, Bay of Chaleur, N.B., is dredged on a regular basis because of high siltation rates. Harbour sediments are anoxic due to the continual input of organic material, primarily wood pulp process wastes from the New Brunswick International Paper Company. Sediments adjacent to an ore-loading facility (Brunswick Mining and Smelting Corporation) contain high concentrations of cadmium (5-10 mg kg^{-1}), lead and zinc. Cadmium present as a contaminant in the lead and zinc ore that is loaded at the facility enters the harbour by accidental spillage during loading, surface runoff or as wind-blown dust from ore stock piles (MacKnight, 1981; 1985).

Chemical analyses suggest a strong correlation between concentrations of total organic carbon and the metals, cadmium and zinc (MacKnight, 1985). Beginning in 1972, sediments dredged from Dalhousie Harbour were dumped 15 km away at an open-water site near Heron Island. By 1978 cadmium concentrations in the harbour sediments (1-2 mg kg^{-1}; MacKnight, 1981) exceeded the Ocean Dumping Control Act (ODCA) allowable limit of 0.6 mg kg^{-1} (Canada Gazette, 1975) and the site was closed. Since then, dredged spoils have been deposited in shoreline containment facilities (MacKnight, 1981).

Evidence of high cadmium (0.88 mg kg^{-1}), lead (53 mg kg^{-1}) and zinc (579 mg kg^{-1}) concentrations in sediments from the dumpsite sampled in 1978, was documented in a study by MacKnight and Schafer (1980). A large proportion of the metals present was bound in organic complexes (mostly wood and bark fibers), with approximately 40% deemed biologically available based on experimental release under weak acid conditions. Macrobenthos were relatively rare at the dumpsite. Most of the organisms present were characteristic of highly contaminated environments. Geochemical surveys were repeated in 1980 (MacKnight, 1981) and 1981 (geochemistry and biota; Packman et al., 1984). While there was a reduction in metal concentrations at the dumpsite center (especially in the surficial sediment where a thin layer of relatively clean sediment had been placed over the old dredged spoils), benthic community structure and species diversity remained altered at the dumpsite (Packman et al., 1984; Tay, 1987).

Marine sediments are the primary sites of nutrient regeneration, organic matter degradation and trace metal transformation (Haines et al., 1982; Lee et al., 1985; Degobbis et al., 1986). Since ecologically vital processes are primarily mediated by microorganisms, measurement of bacterial enzyme activity should be part of any environmental impact assessment. As part of a biogeochemical survey the Heron Island dumpsite was evaluated ten years after its closure. Levels of microbial exoenzyme activity in the sediments were quantified to assess the "recovery".

Studies of the effect of ocean disposal on sediment microbiota have focused on changes in oxygen demand (Phoel et al., 1981) or introduction of foreign bacteria, especially fecal coliforms from sewage sludge disposal (Graikoski, 1981). These effects tend to be relatively short-lived. For example, Phoel et al. (1981) found that oxygen consumption rates returned to normal levels in as little as four days following a disposal event. However, the effect of pollutants on microbial enzyme systems in the aquatic environment can be long-lasting, adversely affecting environmentally-crucial processes. For example, Griffiths et al. (1982a, 1982b) reported that exposure to crude oil caused the cessation of nitrogen fixation in sediments, significant reductions in nitrogen cycling processes for more than 18 m, and a reduction in the rates of organic matter degrading enzymes (chitobiase, cellulase, laminarinase and phosphatase).

Microbial enzyme activity in sediments contaminated by dredged spoils can be compared with those found in adjacent uncontaminated areas. It is suggested that long-term environmental impact at an ocean disposal site can be determined using this approach, even if pre-disposal enzyme activity rates are not known (Lee et al., 1990).

Bacteria are unable to assimilate large macromolecules directly, they must enzymatically cleave them outside the cell before uptake. The activity of bacterial extracellular enzymes (exoenzymes induced by the presence of a suitable substrate outside the cell) can be measured by exposing the bacteria to a low concentration of a fluorogenic substrate analogue. Induced exoenzyme activity can thus be measured without significantly enhancing the natural concentration of a substrate. Since there is a wide range of available fluorogenic substrate analogues, suitable enzyme systems can be chosen given the

conditions at specific study sites.

High concentrations of wood pulp and fibres in the Dalhousie sediment (MacKnight and Schafer, 1980) point to the importance of cellulase activity in the recovery of the Heron Island dumpsite. In the present study fluorogenic substrates specific for the enzymes cellulase (4-methylumbelliferyl cellobioside), phosphatase (4-methylumbelliferyl phosphate) and protease (L-Leucine-4-methylcoumarinyl-7-amide HCl) were used to evaluate the impact of contaminated sediments on biogeochemical processes at the dumpsite.

In response to regulatory requirements of the Canadian Environmental Protection Act (CEPA; Canada Gazette, 1988), Environment Canada is now proposing the use of standard bioassay tests on dredged spoils before disposal in open-water dumpsites. The toxicity of Dalhousie Harbour sediments is not known. In the present study, toxicity tests with Dalhousie Harbour sediments were conducted using new procedures based on the measurement of microbial exoenzyme activity (Lee et al., 1987; 1990).

2.0 MATERIALS AND METHODS

2.1 Field Procedures

For assessment of microbial activity at the Heron Island dumpsite, a grid of 17 stations chosen by Packman et al. (1984) was sampled together with an additional 7 stations including 3 "control" sites (Stns 34, B & C) several kilometers to the east and west of the dumpsite area (Figs 1-2). An additional 8 stations (Stns 1, 5, 7, 8, 11, 13, 16, & 17) were sampled in duplicate (Table 1). Station locations were established using a Del Norte Trisponder System and NAVPAK software with a positioning accuracy of +/- 5 m (McElhanney Systems Ltd., 1988). Sediment samples were collected from a chartered scallop dragger (*M.V. Equinoxe*), using a 0.1 m^2 Van Veen grab sampler. At each station, the enzyme activity of sediment bacteria was measured using fluorogenic substrate analogues specific to the enzymes cellulase, phosphatase and protease.

To assess the toxicity of Dalhousie Harbour sediment, samples were collected at three sites within the harbour, Ferry Wharf (FW), East Wharf (EW) and West Wharf (WW) (Fig 3). The Ferry Wharf is used primarily by the car ferry and small charter craft, while the East and West wharves are used by large cargo (EW) and ore (WW) ships.

2.2 Analysis of Microbial Exoenzyme Activity

2.2.1 Reagents

Synthetic ocean water (SOW) prepared in distilled water according to Morel et al. (1979) was adjusted to pH 8.0 with 1 N NaOH.

Enzyme substrates were prepared as 5 mM solutions of 4-methylumbelliferyl cellobioside, 4-methylumbelliferyl phosphate (Sigma Chemical Co.) or L-Leucine-4-methylcoumarinyl-7-amide HCl (Fluka Chemical Corp.) in 1:10 methanol:SOW (v/v). Working enzyme stocks were then prepared by making 1:32 dilutions in SOW.

A stock methylumbelliferone standard was prepared as a 2.5 mM solution in reagent grade methanol using 4-methylumbelliferone (Sigma Chemical Co.). A working standard solution (1 uM) was prepared by diluting 40 uL of the above standard in 100 mL of SOW.

The NaOH/Borax buffer (pH 10.8) was prepared by mixing with 50 mL of 0.05 M sodium tetraborate with 67 mL of 0.2 M NaOH and adjusting the final volume to 200 mL with distilled water.

2.2.2 Activity Measurement

Microbial enzyme activity was measured in slurries of freshly collected sediment using a modification of the technique described by Meyer-Reil (1986). Sub-samples of sediment from the grab sampler were mixed thoroughly with a glass rod, and replicate aliquots (5) of

approximately 0.2 cm^3 were dispensed into 7 mL polyethylene test tubes using a sterile core tube (4 mm id; 9 mm length). The sediment was suspended in 3 mL of synthetic ocean water (SOW), the tubes were capped, agitated and placed in an incubator for 30 min. Surface water (19°C) was pumped through the incubator to maintain a constant temperature. Following a 30 min preincubation period, 100 uL aliquots of 0.15 mM enzyme substrate solutions were added to each tube and the tubes were incubated for approximately 60 min, with agitation every 20 min. After incubation, the tubes were frozen in dry ice to arrest enzyme activity and transported to the laboratory for fluorescence analysis. Duplicate blanks (SOW and sediment) and standards (1 uM MUF and sediment) were run concurrently. Tubes with sediment were dried overnight in a convection oven (90°C) and weighed to determine the dry weight of sediment in each tube.

To measure the fluorescence of liberated methylumbelliferone (MUF) tubes were thawed in an ice bath and centrifuged at 1450 rpm for 5 min. The supernatant (2.5 mL) was decanted into a glass cuvette and 0.25 mL of the NaOH/Borax buffer was added to maximize the fluorescence of the liberated MUF. Fluorescence was quantified at 455 nm emission with 365 nm excitation, using a Perkin-Elmer MPF-44B fluorescence spectrophotometer. The MUF concentration in each tube was calculated by comparison of the fluorescence to a standard curve prepared using known dilutions of a 1 uM MUF standard solution.

2.2.3 Toxicity Measurement

The toxicity of sediments from Dalhousie Harbour was assessed using a modification of the bioassay developed by Lee et al. (1990). Sediments collected from Stn C were designated "control", while harbour sediments were designated "toxicant". Samples of known volume were dispensed into glass jars using sterile core tubes (made by cutting off the ends of 10 and 30 mL syringes). For the toxicity test, harbour sediments were added to control sediment at dosages of 0, 5, 10, 25, 50, 75 and 100% (v/v) and mixed thoroughly with a glass rod. Aliquots (0.2 cm^3) were then dispensed in triplicate into 7 mL plastic test tubes. Enzyme activity in the tubes was measured by the procedure outlined in section 2.2.2.

Blanks (SOW and sediment) and standards (1 uM MUF and sediment) were run concurrently. Samples (1.0 cm^3) from the bioassay preparations were frozen and returned to the laboratory for dry weight measurement after drying overnight in a convection oven (90°C). Toxic sediment dosages based on dry weight were calculated.

Since bacteria in both the control and harbour sediment contribute to the total enzyme activity, to estimate microbial activity rates for impacted Stn C sediment, the total activity value was corrected by subtracting the product of the activity of the 100% harbour sediment tubes and the dosage (%) of harbour sediment for each assay tube.

3.0 RESULTS

Experimental results from the exoenzyme assays were plotted as topographical and three-dimensional surface figures using the Golden Graphics System (Golden Software Incorporated). This integrated package transforms irregularly spaced data into a regularly spaced grid of data points using a Kriging algorithm and constructs a three-dimensional model based on the data. A combination of both topographical and three-dimensional representations of the data best facilitated interpretation.

Statistical analyses were computed using SYSTAT (Wilkinson, 1987). A BASIC program written to convert the bioassay results from total activity to the activity of the impacted control site sediment is presented in the Appendix.

3.1 Dumpsite Enzyme Activity

Enzyme activity was quite uniform (Table 2) within the general vicinity of the dumpsite. Protease activity was approximately two-fold higher than that reported for cellulase. Phosphatase activity was typically an order of magnitude higher than that found for protease or cellulase. Cellulase activity at the dumpsite center (Stn 7) was significantly different ($P < 0.05$) from 15 of the 32 samples tested and was not different from any of the

control stations (34, B & C). Along the NW-SE transect (Stns 1, 4, 7, 13, 12 & 16), cellulase activity was highest at Stn 7, the dumpsite center (Fig 4). However, since elevated rates of cellulase activity were also detected at other locations in the sampling grid, a statistically significant trend in cellulase activity with increasing distance from the dumpsite center was not observed (Fig. 5). Topographical and three-dimensional representations of cellulase activity indicated that while activity was generally low in the area surrounding the dumpsite it was quite high (<5 uM/g/h) to the west of the dumpsite (Figs 6-7).

Phosphatase activity at the dumpsite center (Stn 7) was significantly lower ($P < 0.05$) than at control stations (Stns 34, B & C). On the NW-SE transect, phosphatase activity was lower over the dumpsite region, with the lowest values at the two stations immediately to the southeast of Stn 7 (Fig 8). A significant correlation between increasing phosphatase activity and increasing distance from the dumpsite was observed. The topographical model (Figs 10-11) showed that phosphatase activity was quite low in the area surrounding the dumpsite with the lowest point southeast of the dumpsite center. Consistent with the results of cellulase activity measurements, the area to the west of the dumpsite had the highest enzyme activity.

In comparison to phosphatase, protease activity was also lower ($P < 0.05$) at the dumpsite center than at the three control stations. Since the values of protease activity at Stn 7 were lower than observed in surrounding stations on the NW-SE transect and much lower than those at distant stations (Fig 12), a positive correlation between protease activity and distance from the dumpsite center was evident (Fig 13). The three-dimensional plots of protease data clearly demonstrated the suppression of activity in the central region of the dumpsite. There was a strong positive correlation between cellulase and phosphatase activity (Fig 16) and between protease and phosphatase activity (Fig 17). Although there was a significant correlation between cellulase and protease activity (Fig 18), this correlation was much weaker than others.

Enzyme activity in duplicate samples (8 of 24 stations) was not significantly different in approximately half of the stations evaluated (Table 3). Although grab sample separation between duplicate samples varied from 5 to 54 m, there did not appear to be a correlation

between grab separation distance and differences in enzyme activity. Differences between replicates may be attributed to the inadvertent assay of sediment subsamples from different depths (an error associated with grab sampling); enzyme activity is known to decrease rapidly within the first few centimeters below the sediment surface (Meyer-Reil, 1986; Lee et al., 1990). We recommend the use of a box core for sampling, in future studies.

Results of inorganic and organic carbon analyses by the OceanChem Group (1988) were compared to our exoenzyme assay results and previous geochemical data collected by Packman et al. (1984). Since the sample digestion methods differed between the 1981 and 1988 surveys (R. Keen, OceanChem Ltd., pers. comm.), the chemical data set for metals between the two surveys could not be compared directly.

Three-dimensional plots of 1988 data indicate that cadmium concentrations were relatively low and quite uniform over most of the area to the west of the dumpsite, however, very low concentrations in the northeast and southeast corners of the dumpsite grid resulted in a ridge of higher concentrations running eastward from the dumpsite (Figs 19-20). Similar patterns were also apparent for lead (Figs 21-22) and zinc (Figs 23-24) concentrations throughout the grid. None of the metals assayed showed significant correlations with distance from the dumpsite center (Figs 25-27). The three-dimensional plots of total inorganic carbon data (TIC) (Figs 28-29) showed elevated concentrations in the area surrounding the dumpsite center and very high concentrations to the southwest of the dumpsite grid. Concentrations of total organic carbon (TOC) were relatively low and uniform in the immediate dumpsite area and elevated to the west and northwest (Figs 30-31). A significant correlation between TIC or TOC concentrations and the vector distance from the dumpsite was not observed.

Cellulase activity was positively correlated with the potential toxic metals of concern (Figs 32-34) while protease and phosphatase were significantly correlated only with lead (Figs 35-40). There was no significant correlation between cellulase, protease and phosphatase activity and either TIC or TOC (Figs 41-46). Correlations for all parameters measured are summarized in Table 4.

The three-dimensional figures constructed with the 1981 data of Packman et al. (1984) showed strikingly different trends from that observed in 1988. Cadmium concentrations were relatively low and uniform in the area surrounding the dumpsite in 1988, while two large peaks of cadmium concentrations to the west of the dumpsite area were apparent in 1981 (Figs 47-48). With the exception that the highest concentrations of lead were observed to the southwest of the dumpsite center, the distribution of lead in 1981 followed a similar pattern to that of cadmium (Figs 49-50). Zinc concentrations were low over the entire dumpsite area except for a large peak to the west of the dumpsite center (Figs 51-52). Observed changes in the trace metal data support the suggestion by MacKnight (1981; 1985) that large amounts of metals are being transported away from the dumpsite on the predominantly westward bottom currents associated with fine suspended particulates.

3.2 Harbour Sediment Toxicity

Most of the bioassays conducted illustrated the toxic effects of harbour sediment on "control" site microbiota. EC_{50} values (the concentration that results in a 50% decrease in enzyme activity) were calculated from a regression of percent effect on harbour sediment dosage, using the linear portion of the curve. Bioassays showed depression of cellulytic activity upon addition of Ferry Wharf and West Wharf sediments (Fig 53) with the Ferry Wharf sediments appearing to be more toxic, yielding an EC_{50} value approximately half that of the West Wharf sediment (Table 6). Since East Wharf sediment stimulated cellulase activity, EC_{50} values could not be calculated.

The toxicity tests based on phosphatase activity demonstrated that sediments from the East Wharf were slightly more toxic than West Wharf sediment (Table 6), while Ferry Wharf sediments appeared to be stimulatory (Fig 54).

Sediments from all three harbour stations inhibited protease activity (Fig 55). From the EC_{50} values it is apparent that protease activity was the most sensitive of the three enzymes assayed (Table 6). East and West Wharf sediments were approximately equal in toxicity while the Ferry Wharf sediment was considerably less toxic. Of the Dalhousie Harbour

sediments evaluated, only those from the West Wharf were consistently toxic, although EC_{50} values were not low, except for protease.

The variation in enzyme activity for the control tubes between experiments conducted over the 2 d period was obvious in the bioassay results. Lowest enzyme activity in uncontaminated control tubes was observed in the toxicity tests conducted with Ferry Wharf sediment using 5 h old control sediment. Enzyme activity of control sediments of the East Wharf and West Wharf toxicity tests was higher since the assays were conducted with reference control sediment collected 18 h and 24 h prior to the experiments. During this period, sediment was stored on deck in a plastic bucket. Enzyme activity in the sediment was probably altered in response to changes in temperature and water circulation.

4.0 DISCUSSION

4.1 Dumpsite Recovery

Results of the enzyme assays which showed stimulation of cellulase activity and corresponding suppression of phosphatase and protease activity in the vicinity of the dumpsite, relative to surrounding regions, suggest that dredged spoils on the ocean dumpsite have a persistent effect. Enhancement of cellulase activity was most likely due to the elevated concentrations of wood pulp and fibers in the dumpsite sediments. Sediment bacteria at the dumpsite appear to have adapted to the high ambient metal concentrations, however, continual degradation of organic matter in the sediment, much of it containing high concentrations of bound metals, may mediate constant input of "free" metals to the biota (MacKnight, 1985). This increase in free metals attributed to the organoclastic enzymes including cellulase may suppress other metabolic pathways. Total metal concentrations do not represent the fraction of metals available to the biota. Statistical analyses indicated that areas of high metal concentrations were also areas with a high potential to degrade cellulose. This supports previous results of MacKnight and Schafer (1980) which indicated a strong correlation between cadmium, zinc and total organic matter concentrations. This association can be illustrated with the stability constants of the metals

with inorganic and organic ligands. The stability constants for cadmium and zinc with an inorganic ligand such as sulfide, common in anoxic harbour sediments, are approximately one order of magnitude lower than the stability constant for lead sulfide. Conversely, the stability constants for zinc and cadmium with vanillin, a common degradation product of lignin, are three-orders of magnitude higher than those for zinc and cadmium sulfides (Sillen and Martell, 1964). The absence of a significant correlation between TOC and Zn, Pb or Cd suggests that chemical characteristics of the Dalhousie dumpsite sediments have changed since 1980.

The depression of phosphatase and protease activity around the dumpsite was clearly portrayed by enzyme activity transects and positive correlations between enzyme activity and distance from the dumpsite center. While the activity of cellulase was also depressed around the vicinity of the dumpsite, high cellulytic activity at the dumpsite center prevented a positive correlation with the vector distance from the dumpsite center. Protease and phosphatase activities were correlated with lead which does not usually form a strong association with organic matter in dumpsite sediments (MacKnight and Schafer, 1980). However, this significant correlation of phosphatase and protease activity with lead may be attributed to one point (Stn 12) which had both low lead concentrations and very low enzyme activity rates. Correlations were much weaker when this point was disregarded.

A stepwise multiple regression test indicated that lead concentrations alone do not significantly affect the activity of the enzymes studied (Table 5). Although particle size analyses have not been completed for this study, Packman et al. (1984) reported that the sediment in the depression to the south of the dumpsite center (Stn 12) was primarily composed of gravel and wood chips. Low metal concentrations and enzyme activity may be associated with coarse sediments, since both metal concentrations (Horowitz,1985) and enzyme activity (Lee et al., 1990) tend to be higher in fine sediments. Results of benthic faunal analysis by Pocklington (1988) showed that while species diversity is up since the last study, and the re-establishment of a classic community found in undisturbed environments is underway, areas of the dumpsite dominated by the Cirratulid, Tharyx spp., and the Apistobranchid, Apistobranchus tullbergi, have yet to recover.

While rates of exoenzyme activity and benthic faunal analyses suggest that the dumpsite has not returned to natural background conditions, three-dimensional plots constructed with 1988 data for Cd, Pb and Zn (OceanChem Group, 1988) showed that the reduction in total metal concentrations observed between 1978 and 1980 by MacKnight (1981; 1985) appears to be continuing. This reduction in total metal concentration and the large peaks to the west of the dumpsite (1981 metal data) have been attributed to the resuspension of fine particulate material, with high concentrations of associated metals, and westward transport on the bottom currents (MacKnight and Schafer, 1980). This westward transport appears to have continued since the 1981 survey.

High concentrations of TOC to the west of the dumpsite indicate that the movement of organic matter may operate at a much slower rate than the current-mediated movement of fine particulates and associated metals. This is attributable to the highly refractory nature of the breakdown products of the wood pulp (i.e., lignins), which formed the major input of organic matter to the dumpsite sediments. In addition to high TOC concentrations at the center, in 1981, Packman et al. (1984) reported high TOC concentrations west of the dumpsite. An observed decrease in TOC concentration at the center by 1988 may have been caused by bacterial processes in the sediments evidenced by the high cellulytic enzyme activity.

Low enzyme activity southeast of the dumpsite center corresponds with a ridge of elevated metal concentrations (observed in the three-dimensional plots of 1988 data) running east from the dumpsite center. This ridge is most likely a reflection of the dumping procedures used. In the last four years of the operation (1974-1978), the hopper barge was opened at the marker buoy and towed east until empty, as opposed to previous stationary dumping at the marker buoy (MacKnight, 1985). Thus, the area receiving dredged spoils directly extends some distance to the east of the dumpsite center. When compared to the corresponding three-dimensional plots of metal distribution in 1981 (Packman et al., 1984), the 1988 concentrations of cadmium and zinc in the dumpsite vicinity are similar with the exception that lead appears to have increased. Direct comparisons between metal concentrations in the two sets of data are not valid since different digestion and measurement techniques were employed. The more rigorous digestion procedures used in

the 1988 study may have a more pronounced effect on lead than the other metals (D. Loring, pers. comm.).

4.2 Harbour Sediment Toxicity

The toxicity tests with Dalhousie Harbour sediments conclusively demonstrated their potential toxicity. The East Wharf sediment was anoxic and contained high concentrations of organic matter, especially wood fibre. High concentrations of cellulose contributed to the stimulation of cellulase activity. Stimulation of enzyme induction by high cellulose concentrations, together with relatively high concentrations of liberated cellulase, in this sediment may have masked any toxic effect on the control site microbiota. This point and the great differences in sensitivity between the protease bioassay and those involving the other enzymes illustrate the need to incorporate a wide range of enzyme systems to determine pollutant effects.

5.0 ACKNOWLEDGEMENTS

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6.0 REFERENCES

Canada Gazette (1988) Canadian Environmental Protection Act and Regulations. House of Commons, Canada Gazette.

Canada Gazette (1975) Ocean Dumping Control Act and Regulations. House of Commons. Canada Gazette.

Degobbis, D., E. Homme-Maslowska, A.A. Orio, R. Donazzolo and B. Pavoni (1986) The role of alkaline phosphatase in the sediments of Venice Lagoon on nutrient regeneration. Est., Coastal and Shelf Sci. 22:425-437.

Graikoski, J.T. (1981) Bacteriology of ocean dumping. In: 3rd. Internat. Ocean Disposal Symp. Abstracts., Woods Hole, Mass. p. 54.

Griffiths, R.P. (1983) The importance of measuring microbial enzymatic functions while assessing and predicting long-term anthropogenic perturbations. Mar. Pollut. Bull. 14:162-165.

Griffiths, R.P., B.A. Caldwell, W.A. Broich and R.Y. Morita (1982a) The long-term effects of crude oil on microbial processes in subarctic marine sediments. Est. Coast. Shelf Sci. 15:183-198.

Griffiths, R.P., B.A. Caldwell, W.A. Broich and R.Y. Morita (1982b) Long-term effects of crude oil on microbial processes in subarctic marine sediments: studies on sediments amended with organic nutrients. Mar. Pollut. Bull. 13:273-278.

Haines, J.R., R.M. Atlas, R.P. Griffiths and R.Y. Morita (1982) Denitrification and nitrogen fixation in Alaskan continental shelf sediments. Appl. Environ. Microbiol. 41:412-421.

Horowitz, A.J. (1985) A primer on trace metal - sediment chemistry. U.S. Geological Survey, Water-Supply Paper 2277. 67 p.

Lee, K., K.L. Tay, C.N. Ewing and E.M. Levy (1990) Toxicity and environmental impact assessment tests based on the activity of indigenous bacteria. Ocean Dumping Report 4, Environment Canada. 138 pp.

Lee, K., C.N. Ewing and E.M. Levy (1987) The role of bacterial bioassays in predicting the potential impact of dumping contaminated sediments in the ocean. In: Proc. 7th Internat. Ocean Disposal Symp. Wolfville, N.S., Canada, September 21 - 25, 1987. pp. 540-563.

Lee, K., C.S. Wong, W.J. Cretney, F.A. Whitney, T.R. Parsons, C.M. Lalli and J. Wu (1985) Microbial response to crude oil and Corexit 9527: SEAFLUXES enclosure study. Microb. Ecol. 11:337-351.

MacKnight, S.D. (1981) The Dalhousie ocean dumpsite, 1978 and 1980: A geochemical view of history. In: 3rd Internat. Ocean Disposal Symp., Abstracts., Woods Hole, Mass. p. 87.

MacKnight, S.D. (1984) Ocean disposal of dredged material from Atlantic Canada harbours. 1. Small craft harbours, Scotia/Fundy Region. OceanChem Ltd.

MacKnight, S.D. (1985) Geochemistry of the Dalhousie ocean dumpsite. In: B. Ketchum, J. Capuzzo, W. Burt, I. Duedall, P. Park and D. Kester (eds.), Wastes in the Ocean. Vol. 6: Near-shore Waste Disposal. pp. 281-301.

MacKnight, S.D. and C.T. Schafer (1980) Geochemistry and benthic ecology at the Dalhousie, New Brunswick ocean dump site. In: Proc. 9th World Dredging Conf. (WODCON IX), Vancouver, B.C. pp. 761-781.

Meyer-Reil, L.-A. (1986) Measurement of hydrolytic activity and incorporation of dissolved organic substrates by microorganisms in marine sediments. Mar. Ecol. Prog. Ser. 31: 143-149.

Morel, F.M.M., J.G. Rueter, D.M. Anderson and R.R.L. Guillard (1979) AQUIL: A chemically defined phytoplankton culture medium for trace metal studies. J. Phycol. 15: 135-141.

OceanChem Group (1988) Dredged sediment and ocean disposal site assessment study, Dalhousie, N.B., 1988. Report submitted to Environmental Protection, Environment Canada (Atlantic Region), June, 1988. 26 pp.

Packman, G.A., K.L. Tay and C. Berman (1984) Environmental monitoring at the Heron Island dump site in the Bay of Chaleur near Dalhousie, N.B. Environment Canada Atlantic Region Surveillance Report. EPS-5-AR-84-6. 91 p.

Phoel, W. C., J.M. Wells and S. Spina (1981) In situ investigation to determine the rates of oxygen consumption by sewage sludge on the seafloor. In: 3rd. Internat. Ocean Disposal Symp. Abstracts., Woods Hole, Mass. p. 47.

Pocklington, P. (1988) Heron Island Ocean Dumpsite Survey - 1988. Report submitted to Environmental Protection, Environment Canada (Atlantic Region), September, 1988. 26 p.

Sillen, L.G. and A.E. Martell (1964) Stability constants of metal-ion complexes. The Chemical Society. London. Special Publ. 17. 754 p.

Tay, K.L. (1987) An overview of dredged material ocean dump site monitoring and assessment programs and dump site designation conducted by Environment Canada under the Ocean Dumping Control Act in the Atlantic Region. In: Proc. 7th Internat. Ocean Disposal Symp. Wolfville, N.S. pp. 292-315.

Wilkinson, L. (1987) SYSTAT: The system for statistics. SYSTAT Inc., Evanston Il.

7.0 TABLES

TABLE 1: Sampling station locations (from McElhanney Services Ltd.)

Station	Latitude	Longitude
1	48 03' 49.5"N	66 10' 21.0"W
1(2)	03' 49.4"	10' 20.8"
2	02' 59.5"	11' 24.1"
3	03' 11.9"	10' 35.7"
4	03' 23.9"	09' 38.8"
5	03' 30.1"	08' 47.3"
5(2)	03' 30.1"	08' 49.0"
6	02' 48.7"	10' 55.2"
7	02' 59.9"	09' 38.4"
7(2)	03' 00.2"	09' 38.7"
8	03' 06.0"	08' 41.2"
8(2)	03' 05.9"	08' 40.9"
9	02' 23.7"	11' 00.2"
10	02' 35.8"	10' 18.8"
11	02' 37.4"	10' 05.4"
11(3)	02' 37.2"	10' 05.4"
12	02' 47.1"	09' 28.9"
13	02' 54.1"	09' 34.7"
13(2)	02' 54.0"	09' 32.1"
14	02' 52.8"	08' 35.6"
15	02' 11.4"	10' 29.4"
16	02' 18.0"	09' 04.5"
16(2)	02' 18.6"	09' 05.2"
17	02' 23.6"	08' 12.6"
17(2)	02' 23.9"	08' 11.8"
18	02' 24.6"	09' 59.7"
19	02' 42.5"	10' 00.3"
27	03' 08.5"	10' 47.7"
33	02' 44.8"	11' 14.5"
34	02' 33.0"	13' 44.8"
B	04' 08.1"	15' 33.1"
C	00' 01.2"	02' 38.9"

TABLE 2: Enzyme activity (uM/g/h) at dumpsite stations. 25 - 28 July, 1988.

STATION	CELLULASE		PHOSPHATASE		PROTEASE	
	Act.	S.E.	Act.	S.E.	Act.	S.E.
1	2.52	0.23	103.9	11.3	4.0	0.4
1(2)	2.81	0.19	85.4	5.7	5.8	0.9
2	1.1	0.09	55	4.7	1.54	0.26
3	2.01	0.19	75.6	7.9	2.98	0.8
4	1.19	0.07	53.3	1.9	1.9	0.17
5	0.94	0.06	48.1	3.3	2.49	0.24
5(2)	1.77	0.17	61.1	5.5	7.23	0.79
6	6.22	0.25	124.7	8.2	6.23	1.86
7	2.67	0.36	32.4	4.3	0.87	0.04
7(2)	3.49	0.27	70.1	2.2	1.9	0.3
8	2.92	0.09	115.3	11.5	4.9	0.55
8(2)	2.97	0.23	60.5	2.7	4.97	0.44
9	2.8	0.11	133.7	15.4	7.36	2.21
10	2.46	0.21	74.4	7.1	5.09	0.86
11	4.78	0.62	75.7	15.4	2.03	0.28
11(3)	2.12	0.12	76.7	7.3	3.75	0.32
12	0.19	0.04	7.9	0.8	0.36	0.03
13	1.34	0.12	37.7	1.4	2.45	0.17
13(2)	1.38	0.10	45.2	3.1	3.13	0.24
14	1.56	0.05	59.7	4.7	3.72	0.23
15	2.56	0.19	91.4	7.9	1.84	0.33
16	2.79	0.13	85.4	4.4	5.08	0.18
16(2)	1.23	0.08	42	2	0.91	0.14
17	1.54	0.09	72	4	2.08	0.20
17(2)	2.68	0.46	83.6	3.1	5.88	0.36
18	3.24	0.44	94.9	11.8	7.99	0.97
19	3.24	0.24	72.7	8	2.26	0.22
27	3.8	0.08	180.1	25.4	6.1	0.53
33	2.89	0.35	96.6	12.6	4.96	0.63
34	2.79	0.37	84.5	4.9	5.34	0.31
B	2.5	0.16	55.3	5.1	4.01	0.29
C	2.98	0.74	78.8	8.7	4.18	0.41

TABLE 3: Comparison of enzyme activity in samples from stations sampled in duplicate.

Station	Grab sample Separation (m)**	Activity (uM/g/h +/- S.E.) CELL	PO4	LEU
1	5	2.5 ±.2	104±11	4.0±.4
1(2)		2.8 ±.2	85± 6	5.8±.9
5	35	0.94±.06*	48± 3	2.5±.2*
5(2)		1.8 ±.2	61± 6	7.2±.8
7	10	2.7 ±.4	32± 4*	0.87±.04*
7(2)		3.5 ±.3	70± 2	1.9 ±.3
8	7	2.92±.09	115±12*	4.9 ±.6
8(2)		2.9 ±.2	61± 3	5.0 ±.4
11	6	4.8 ±.6*	76±15	2.0 ±.3*
11(3)		2.1 ±.1	77± 7	3.8 ±.3
13	54	1.3 ±.1	38± 1	2.5 ±.2*
13(2)		1.4 ±.1	45± 3	3.1 ±.2
16	24	2.8 ±.1*	85± 4*	5.1 ±.2*
16(2)		1.23±.08	42± 2	0.9 ±.1
17	19	1.54±.09*	72± 4	2.1 ±.2*
17(2)		2.7 ±.5	84± 3	5.9 ±.4

CELL = Cellulase Activity (n=5)
PO4 = Phosphatase Activity (n=5)
LEU = Protease Activity (n=5)

* Denotes significant differences between replicate samples ($P<0.05$).

** Position accuracy was approximately +/- 5 m (McElhanney Services Ltd.).

Table 4: Pearson correlation matrix of enzyme activity and chemical (OceanChem Ltd.) data.

	PO4	CELL	LEU	DIST	CD	PB	ZN	TOTMET	TOC	TIC
PO4	1.000									
CELL	0.658*	1.000								
LEU	0.769*	0.516*	1.000							
DIST	0.577*	0.186	0.469*	1.000						
CD	0.278	0.502*	0.143	0.036	1.000					
PB	0.497*	0.633*	0.537*	0.245	0.757*	1.000				
ZN	0.113	0.445*	0.167	-0.157	0.789*	0.687*	1.000			
TOTMET	0.215	0.518*	0.268	-0.067	0.830*	0.807*	0.984*	1.000		
TOC	0.140	-0.159	0.044	0.186	-0.031	-0.258	-0.325	-0.328	1.000	
TIC	0.115	0.415	0.072	0.060	0.400	0.586*	0.550*	0.593*	-0.803*	1.000

* Denotes significant correlations at $P \leq 0.05$. (n = 20)

PO4 = Phosphatase Activity; CELL = Cellulase Activity; LEU = Protease activity; DIST = Distance from Dumpsite Centre; CD = Cadmium Concentration; PB = Lead Concentration; ZN = Zinc Concentration; TOTMET = Total Metal Concentration (Cd+Pb+Zn); TOC = Total Organic Carbon Concentration; TIC = Total Inorganic Carbon Concentration.

TABLE 5: Results of stepwise multiple regressions of enzyme activity data ($p<0.05$). Regressions were computed using SYSTAT software (Wilkinson, 1987). Variables tested are listed below.

Independant Variable	Dependant Variables	r^2 Value
CELL	PO4	0.433
	TOTMET	0.582
PO4	LEU	0.591
	CELL	0.684
	DIST	0.756
LEU	PO4	0.591

CELL = Cellulase Activity
PO4 = Phosphatase Activity
LEU = Protease Activity
DIST = Vector Distance from Dumpsite
CD = Cadmium Concentration
PB = Lead Concentration
ZN = Zinc Concentration
TOTMET = Total Metal Concentration (Cd + Pb + Zn)

TABLE 6: Toxicity of Dalhousie Harbour sediments to Stn C sediment enzyme activity.

	EC_{50} (% Dosage by weight)		
Station	Cellulase	Phosphatase	Protease
FW	32	Stim.	48
EW	Stim.	49	8.5
WW	71	78	9.6

8.0 FIGURES

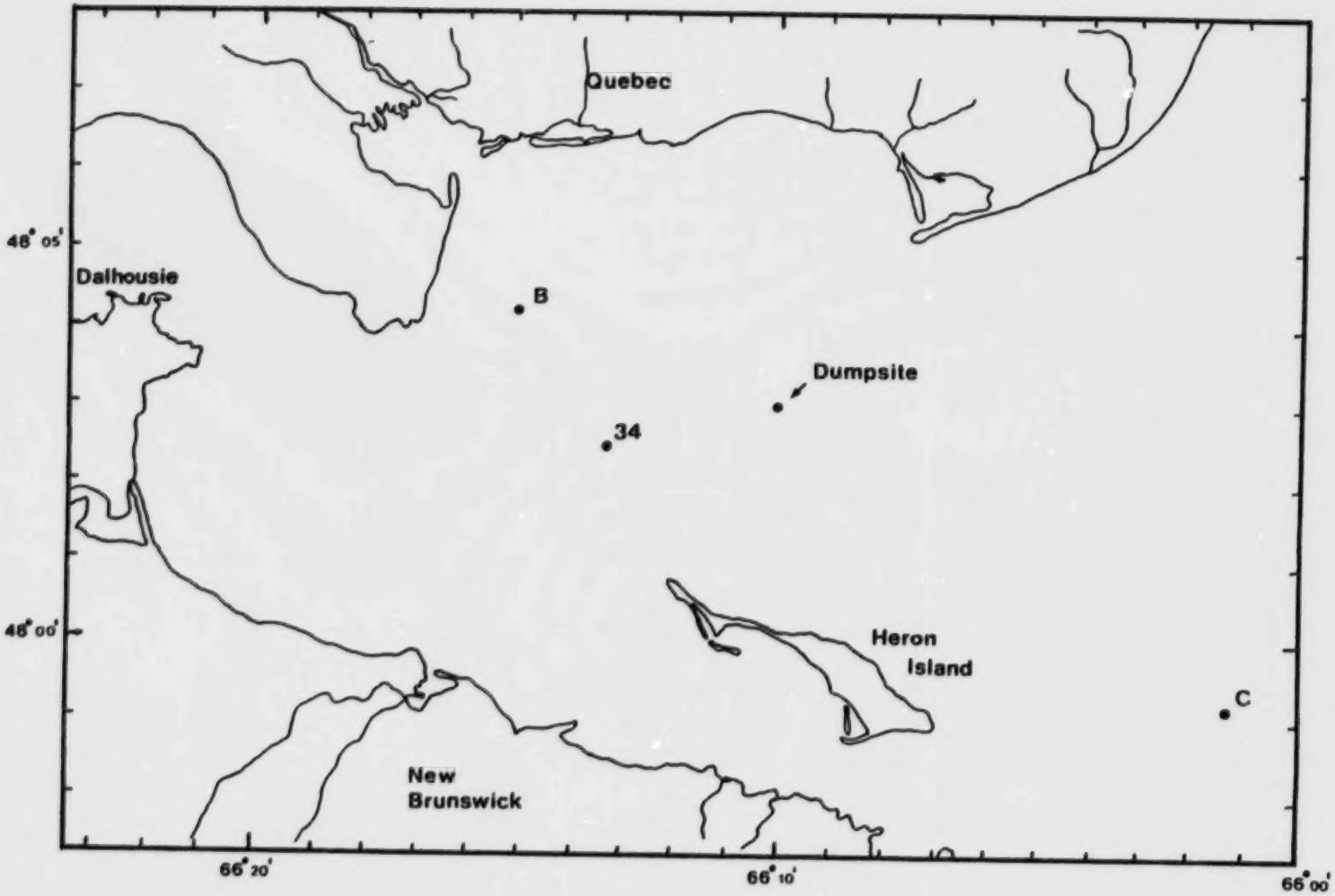


FIGURE 1: Control site and dumpsite loactions in the Bay of Chaleur, New Brunswick.

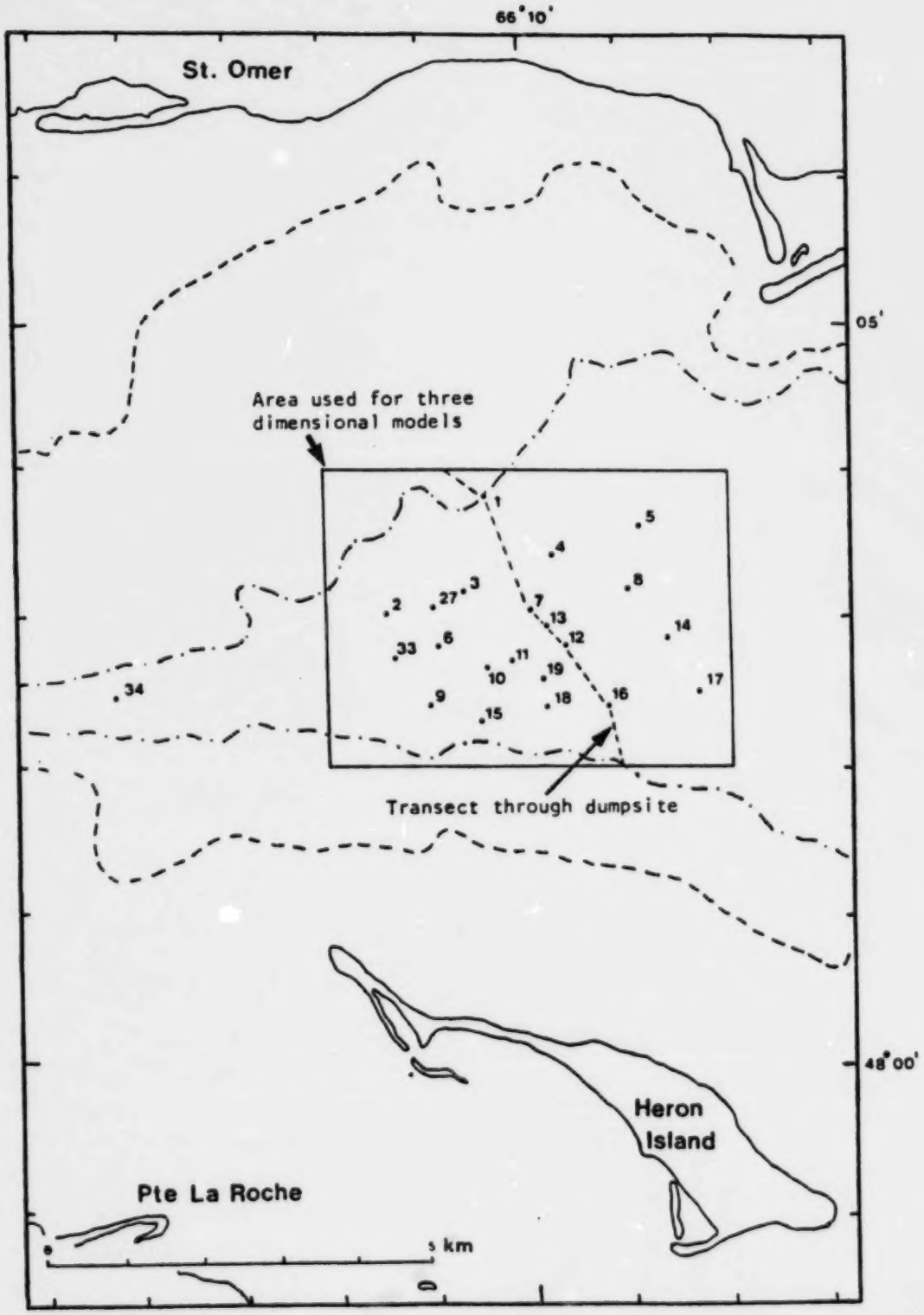


FIGURE 2: Dumpsite sampling area showing sample station locations and NW - SE transect through dumpsite centre.

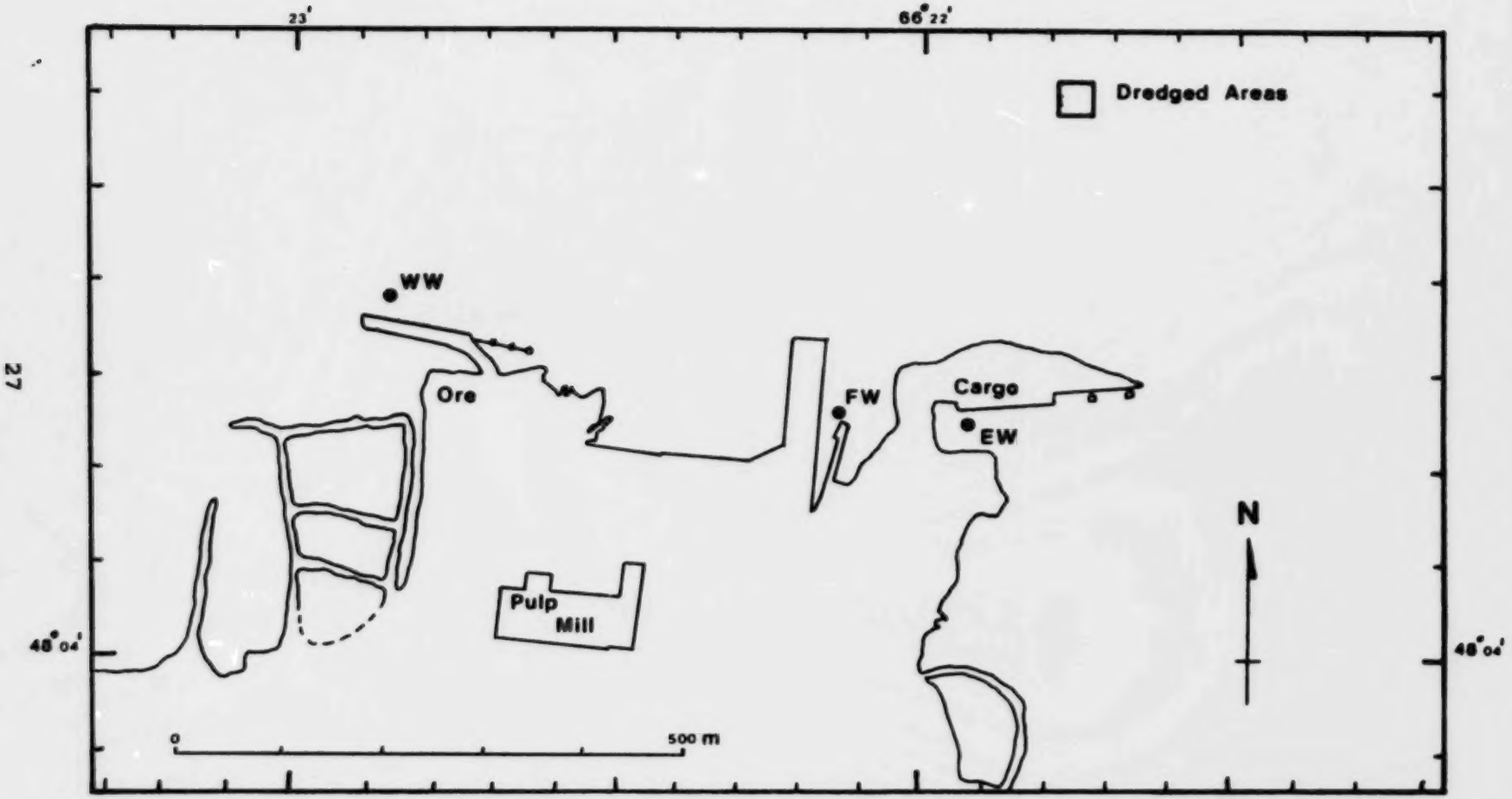


FIGURE 3: Dalhousie Harbour, New Brunswick, showing locations of sampling sites and areas frequently dredged (shaded).

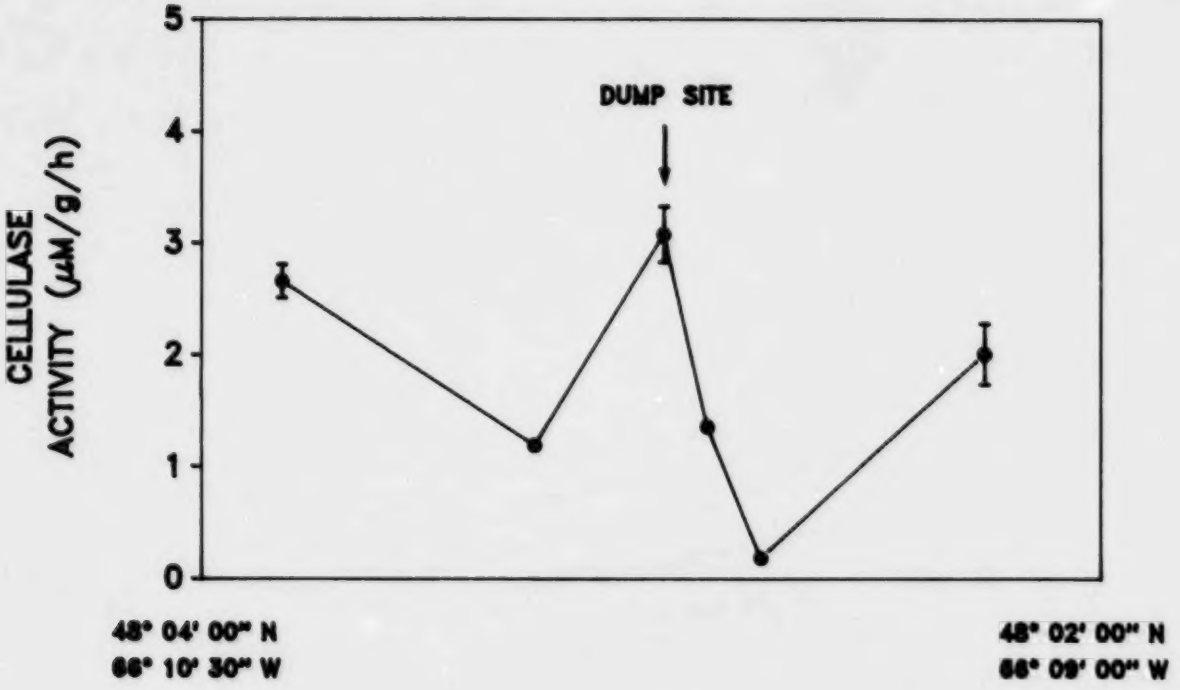


FIGURE 4: Cellulase activity along a NW - SE transect through the dumpsite centre. Values are mean +/- S.E.

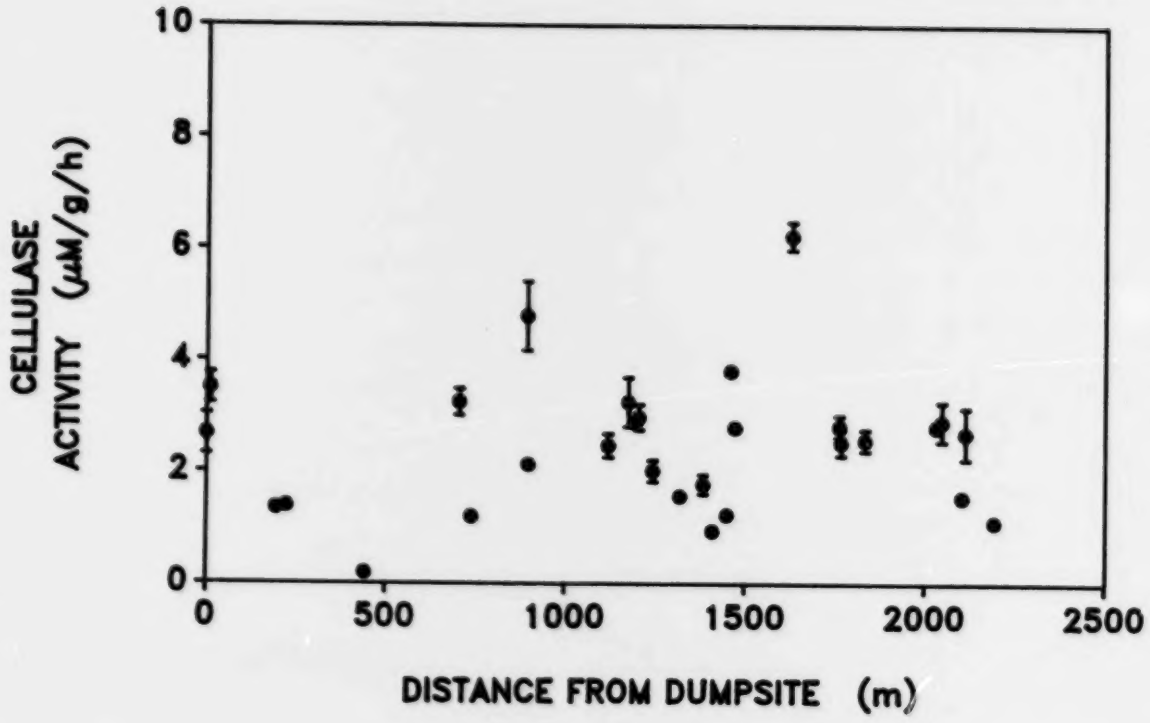


FIGURE 5: Relationship between cellulase activity and vector distance from the dumpsite centre. Values are mean +/- S.E.

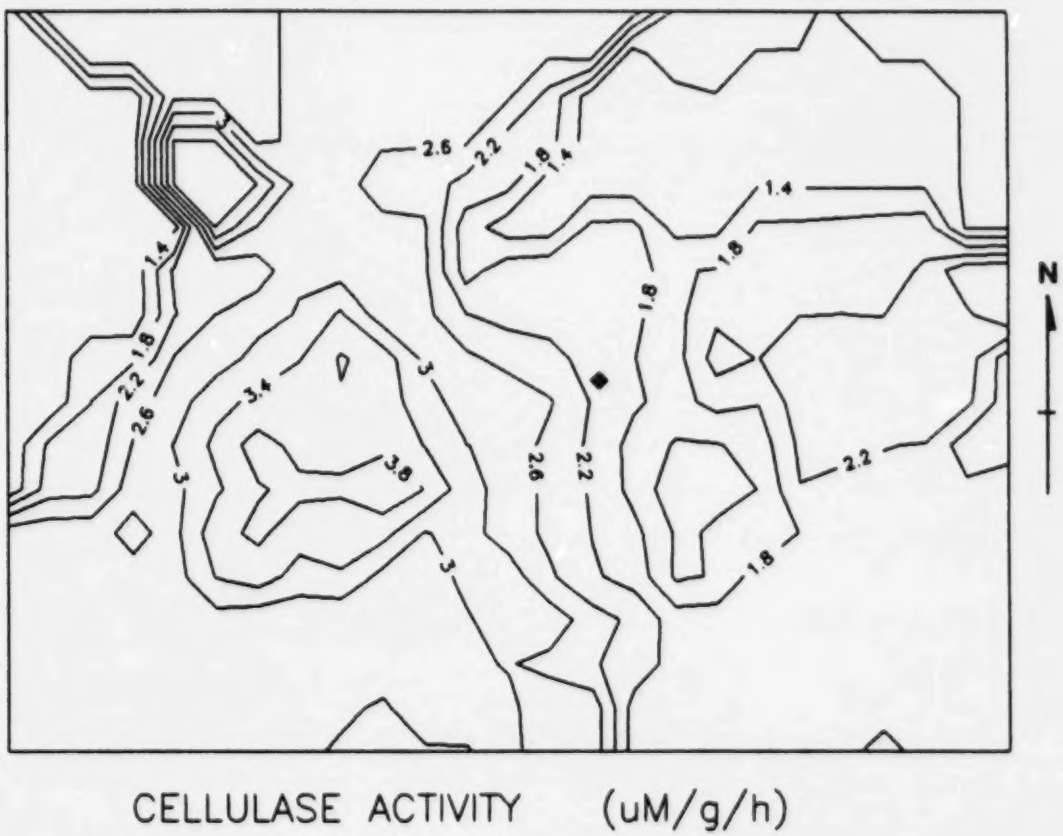


FIGURE 6: Topographical representation of cellulase activity in the dumpsite survey area. Dumpsite centre (◆).

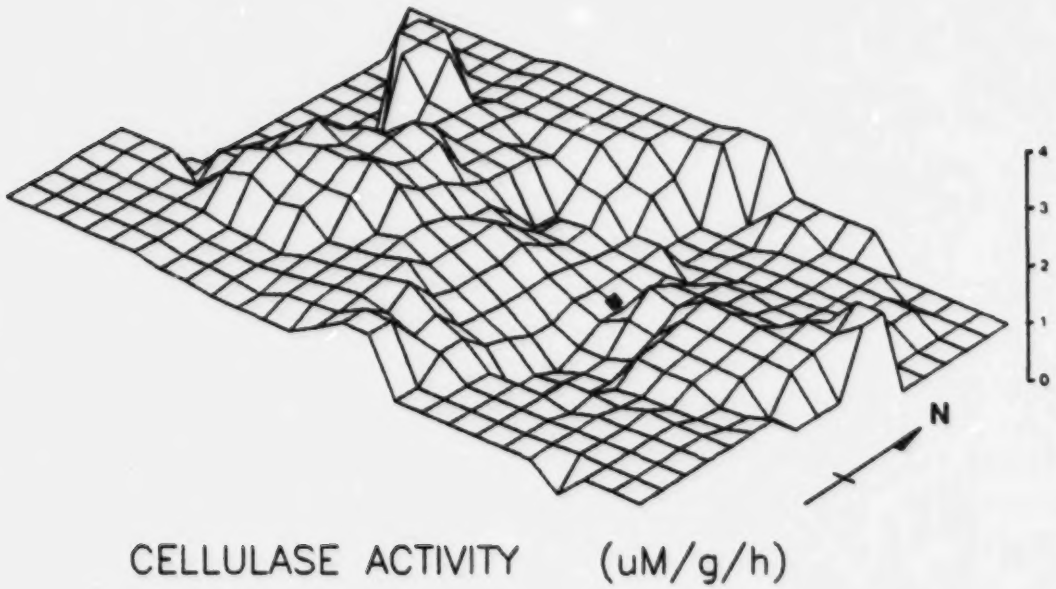


FIGURE 7: Three dimensional representation of cellulase activity in the dumpsite survey area. Dumpsite centre (◆).

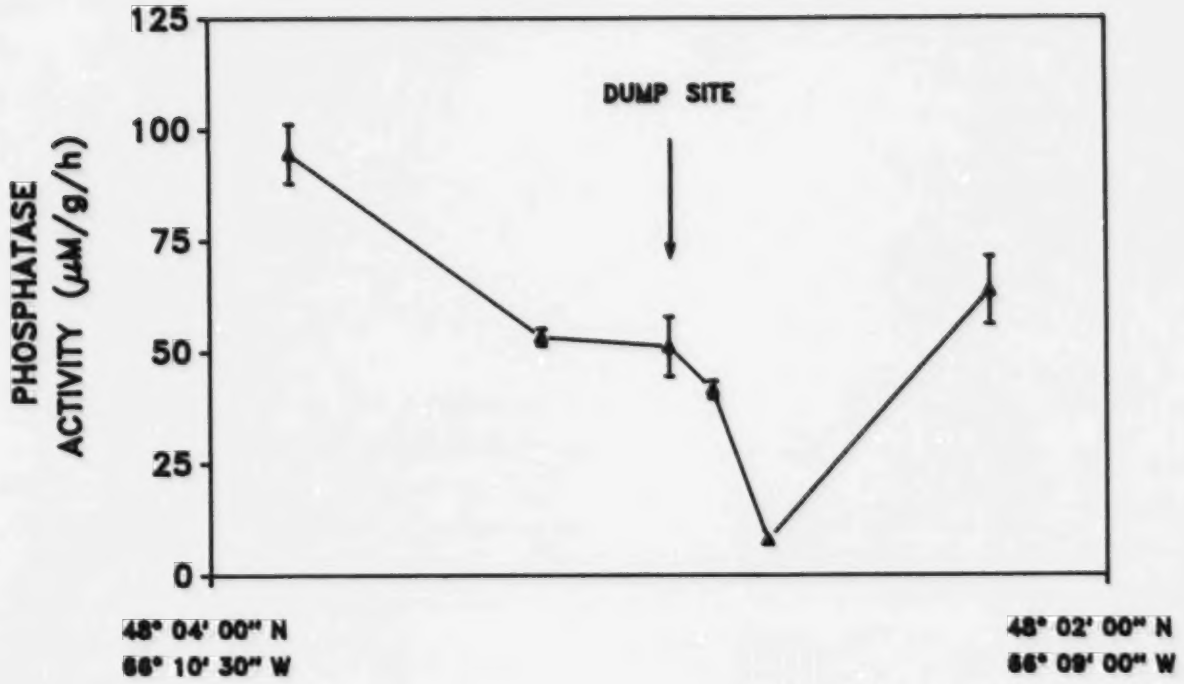


FIGURE 8: Phosphatase activity along a NW - SE transect through the dumpsite centre. Values are mean +/- S.E.

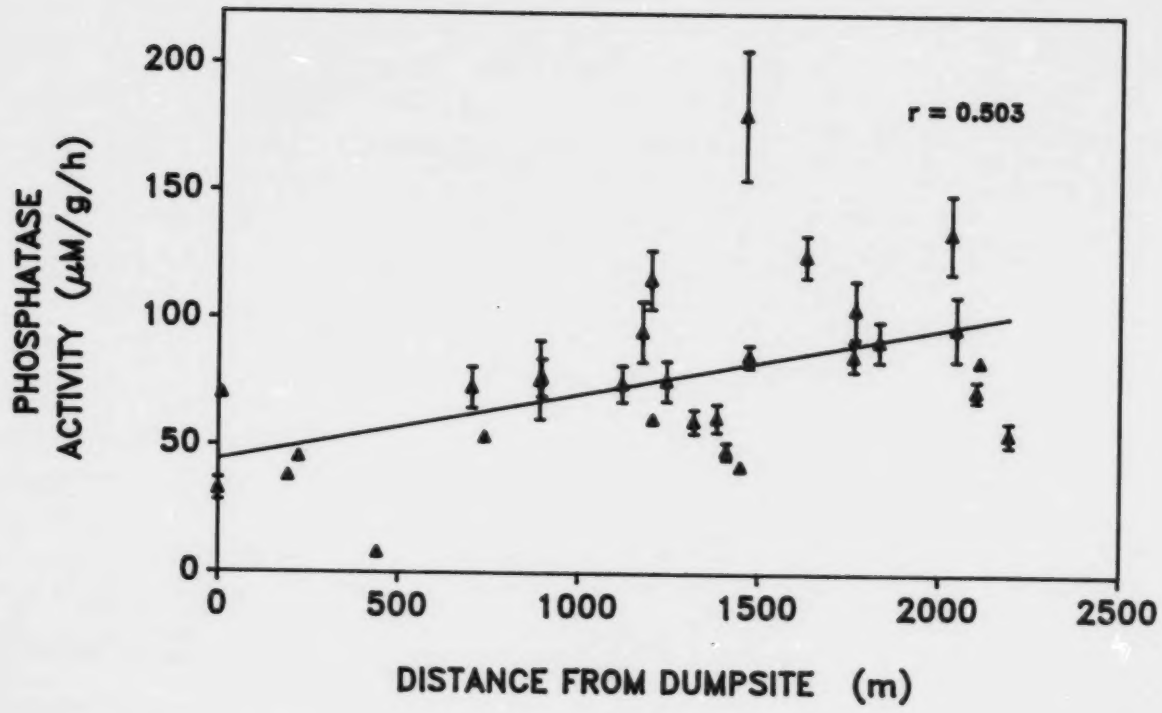


FIGURE 9: Relationship between phosphatase activity and vector distance from the dumpsite centre. Values are mean +/- S.E.

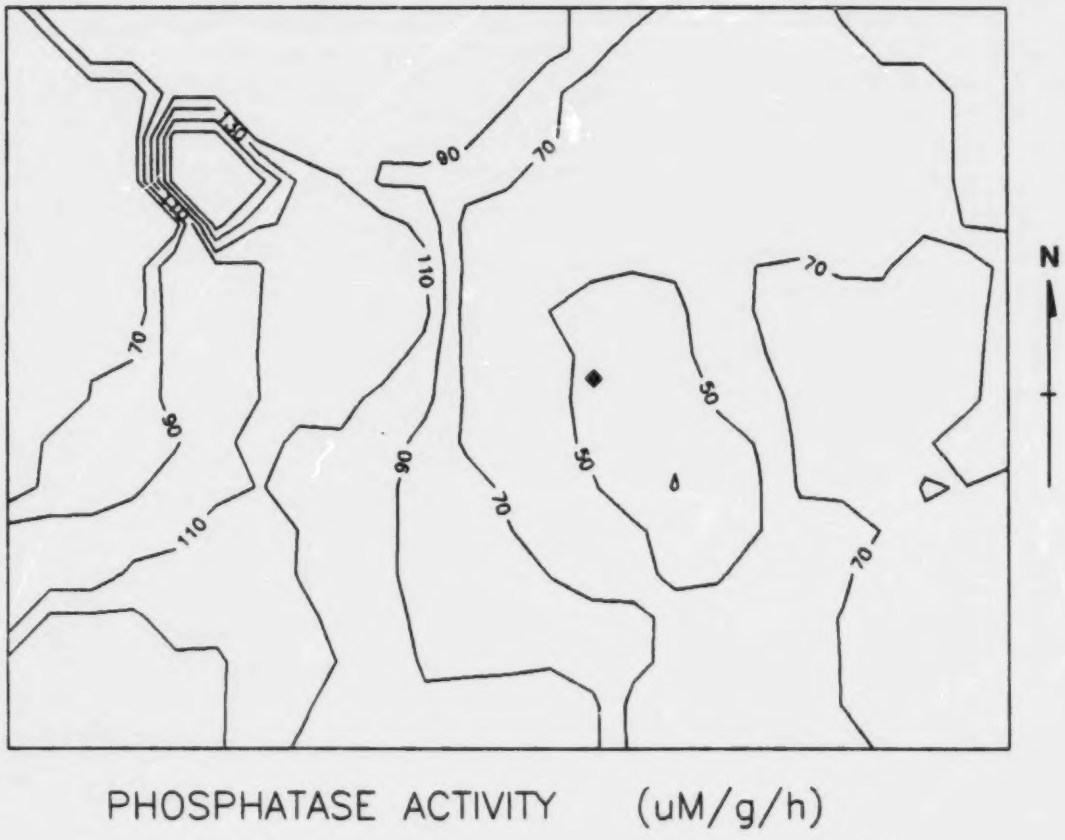
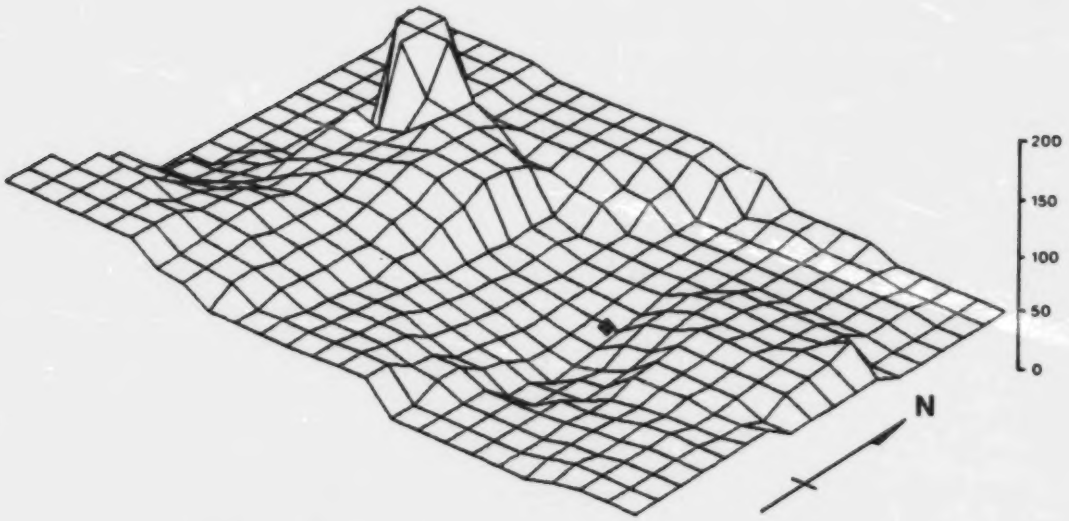


FIGURE 10: Topographical representation of phosphatase activity in dumpsite survey area. Dumpsite centre (◆).



PHOSPHATASE ACTIVITY (uM/g/h)

FIGURE 11: Three dimensional representation of phosphatase activity in the dumpsite survey area. Dumpsite centre (◆).

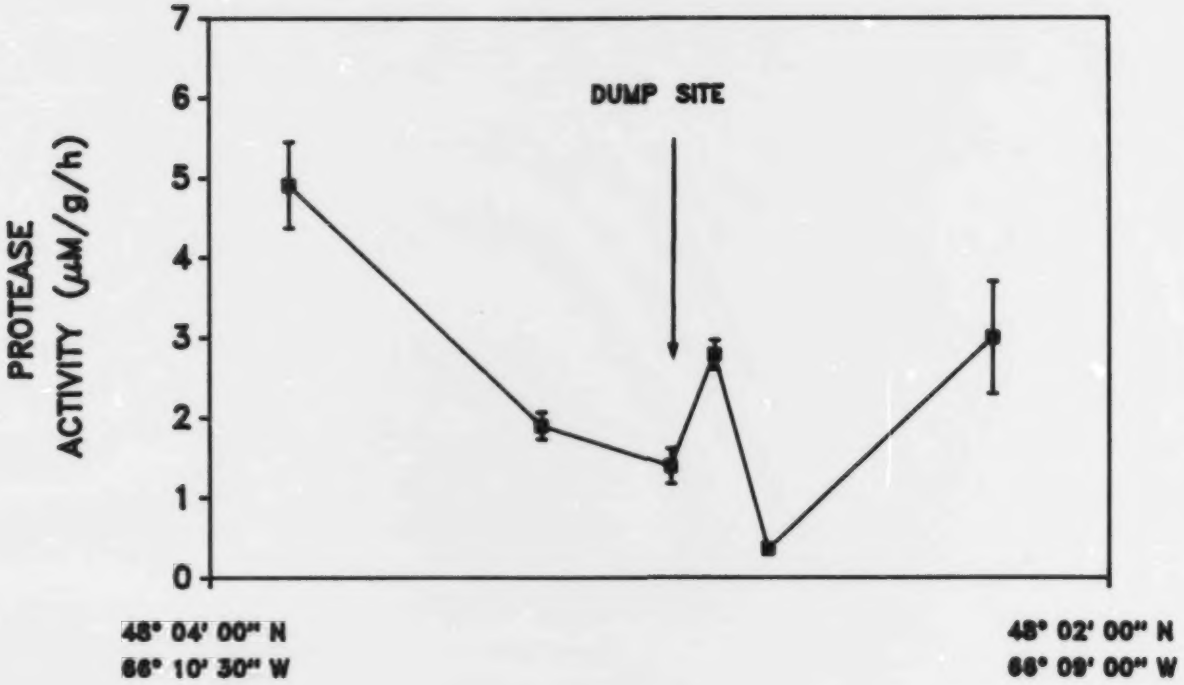


FIGURE 12: Protease activity along a NW - SE transect through the dumpsite centre. Values are mean +/- S.E.

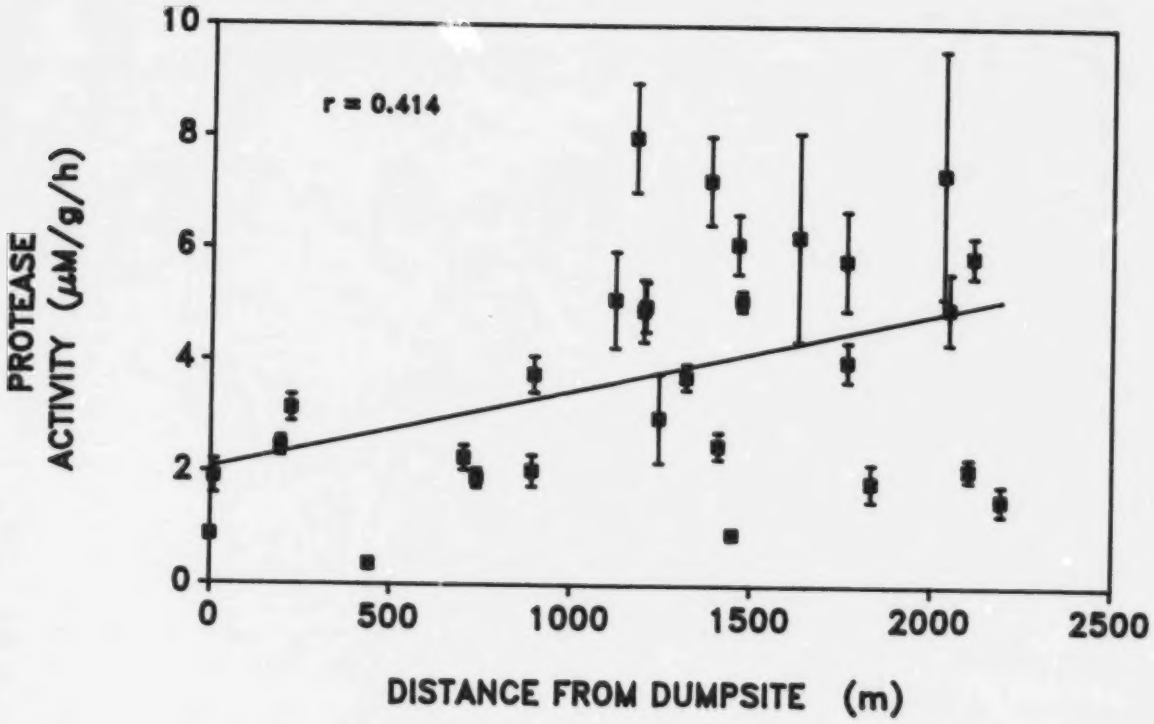


FIGURE 13: Relationship between protease activity and vector distance from the dumpsite. Values are mean +/- S.E.

FIGURE 14: Topographical representation of protease activity in the dumpsite area. Dumpsite centre (◆).

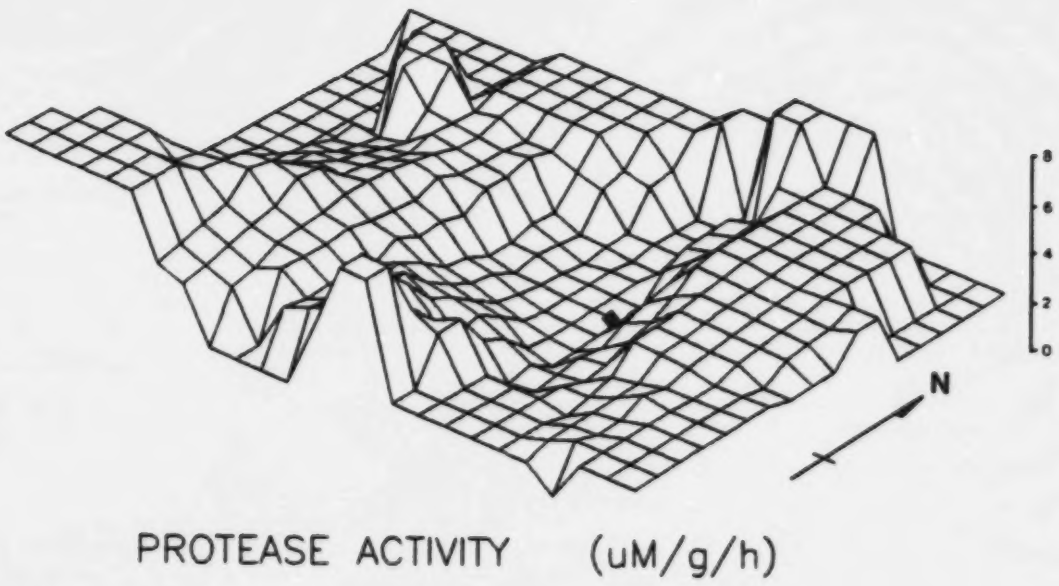


FIGURE 15: Three dimensional representation of protease activity in the dumpsite area. Dumpsite centre (◆).

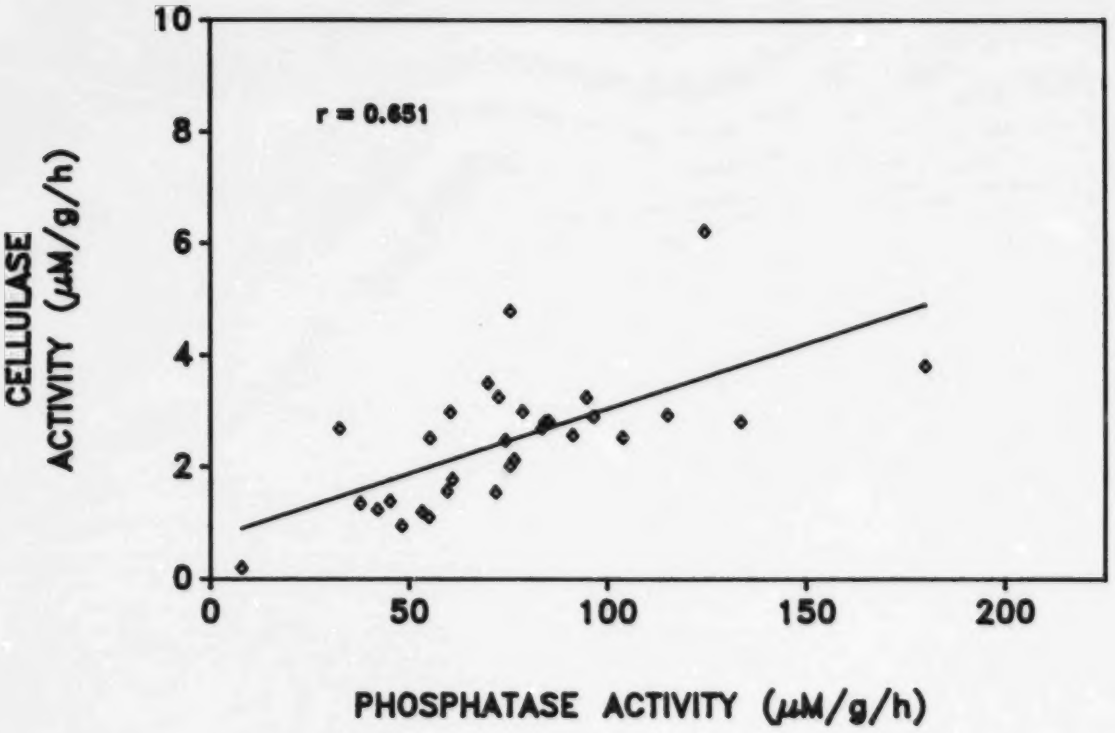


FIGURE 16: Relationship between cellulase and phosphatase activity in the dumpsite sampling area.

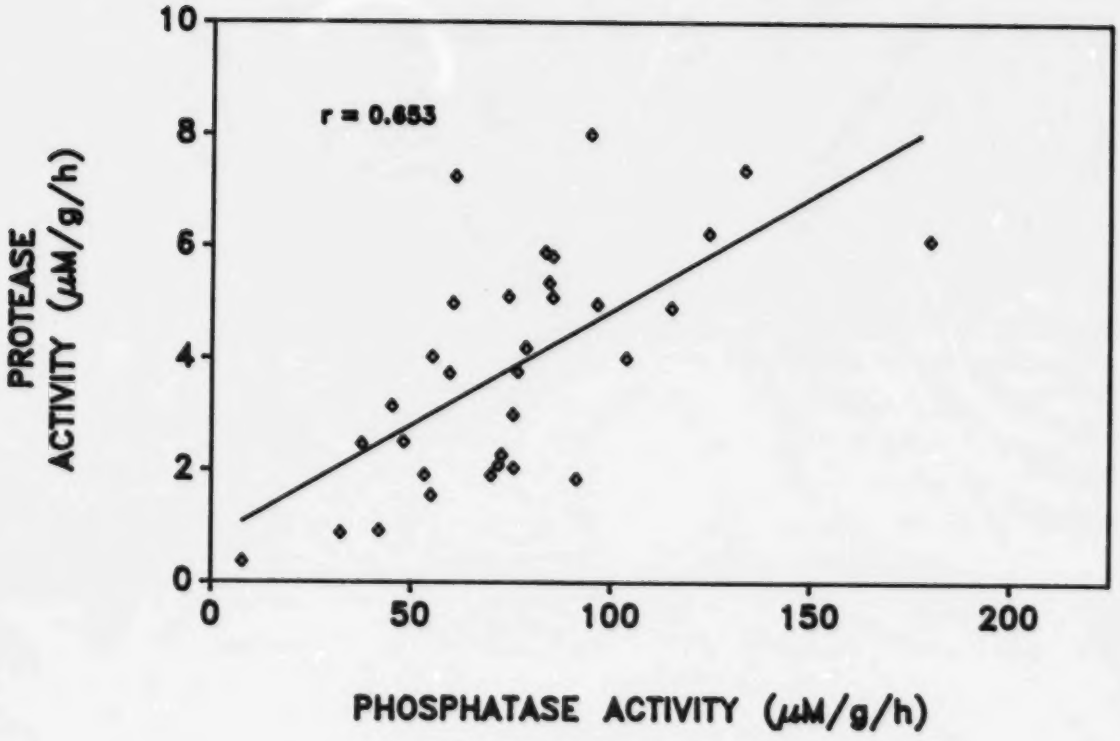


FIGURE 17: Relationship between protease and phosphatase activity in the dumpsite area.

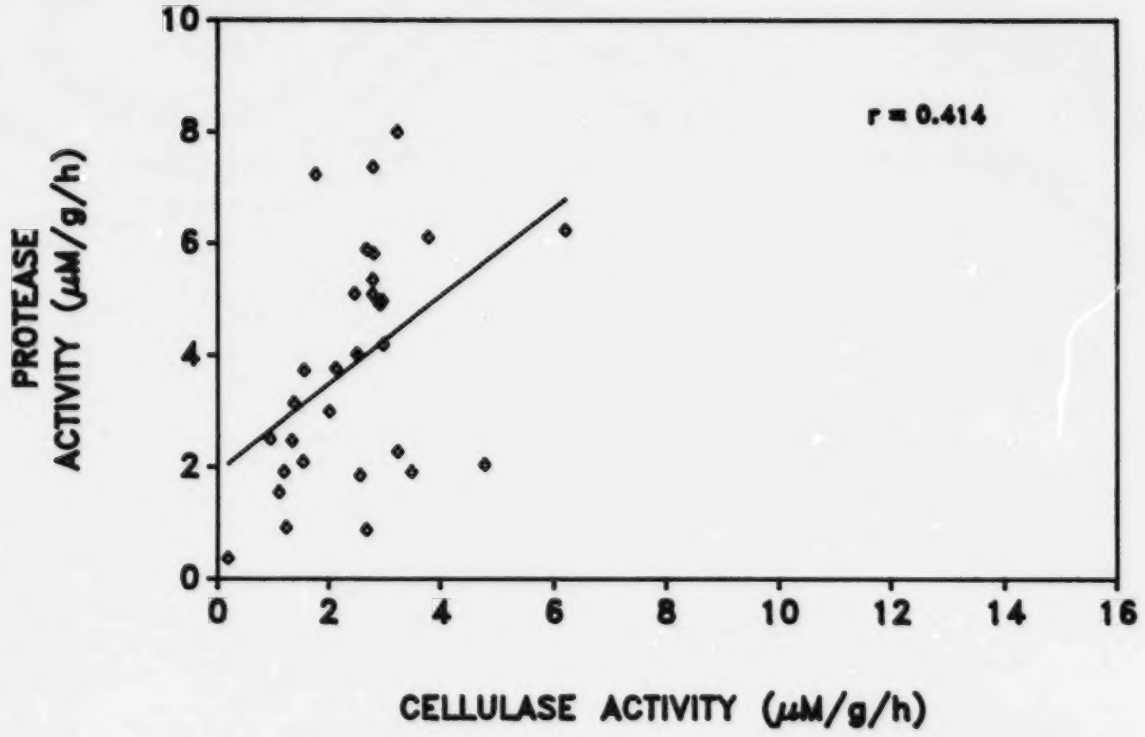


FIGURE 18: Relationship between protease and cellulase activity in the dumpsite area.

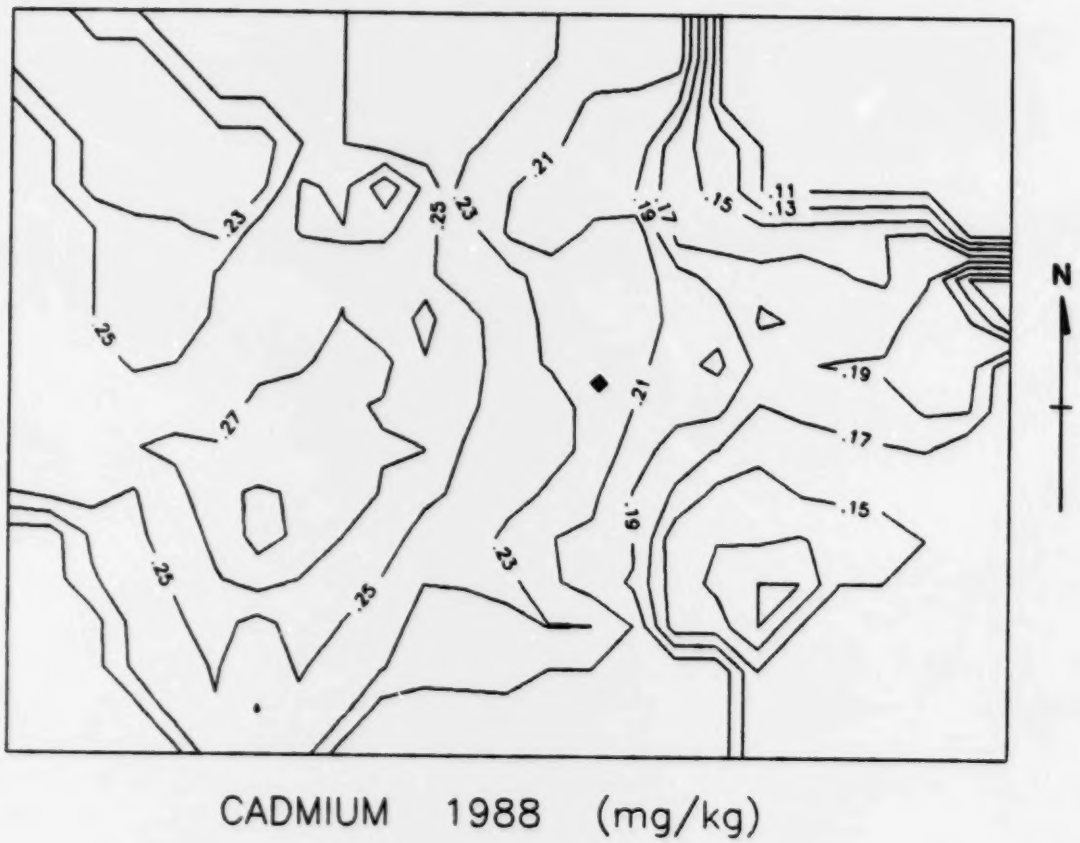
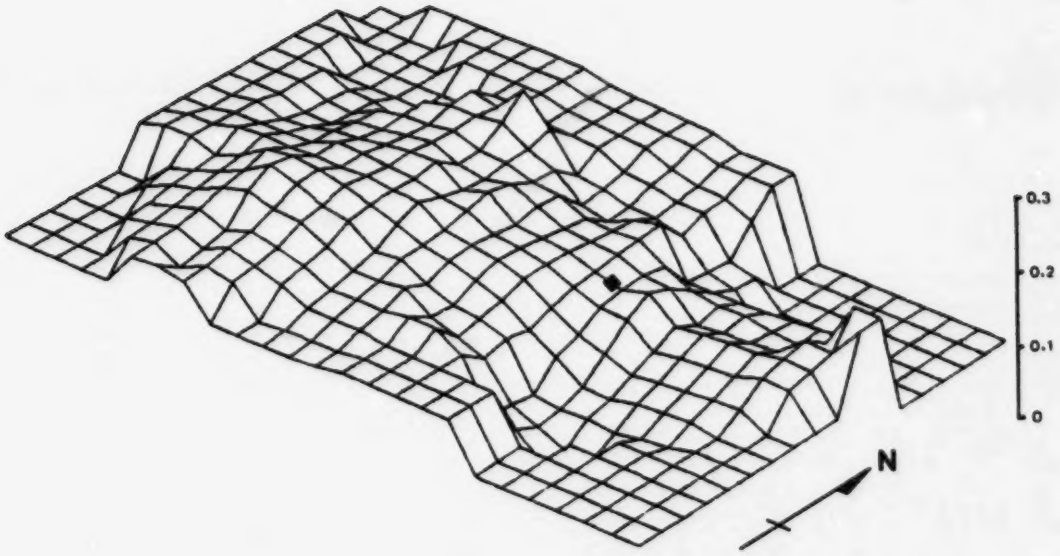


FIGURE 19: Topographical representation of 1988 cadmium data in the dumpsite sampling area. Dumpsite centre (◆).



CADMIUM 1988 (mg/kg)

FIGURE 20: Three dimensional representation of 1988 cadmium data in the dumpsite sampling area. Dumpsite centre (◆).

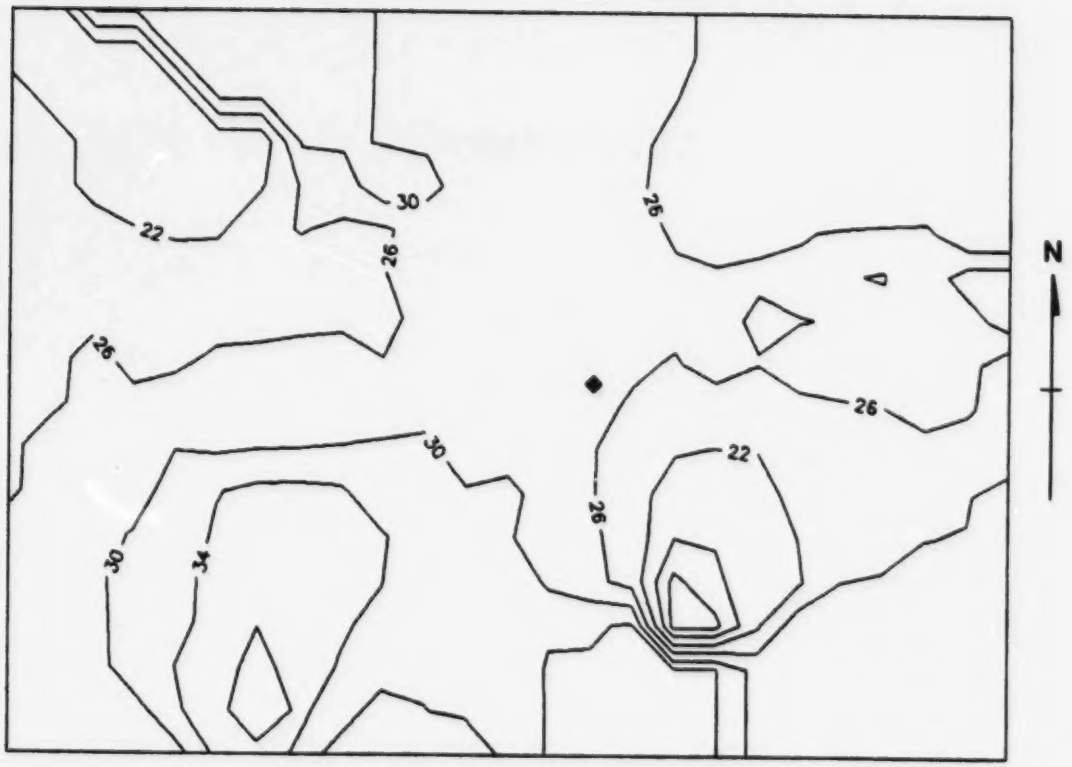


FIGURE 21: Topographical representation of 1988 lead data in the dumpsite survey area. Dumpsite centre (◆).

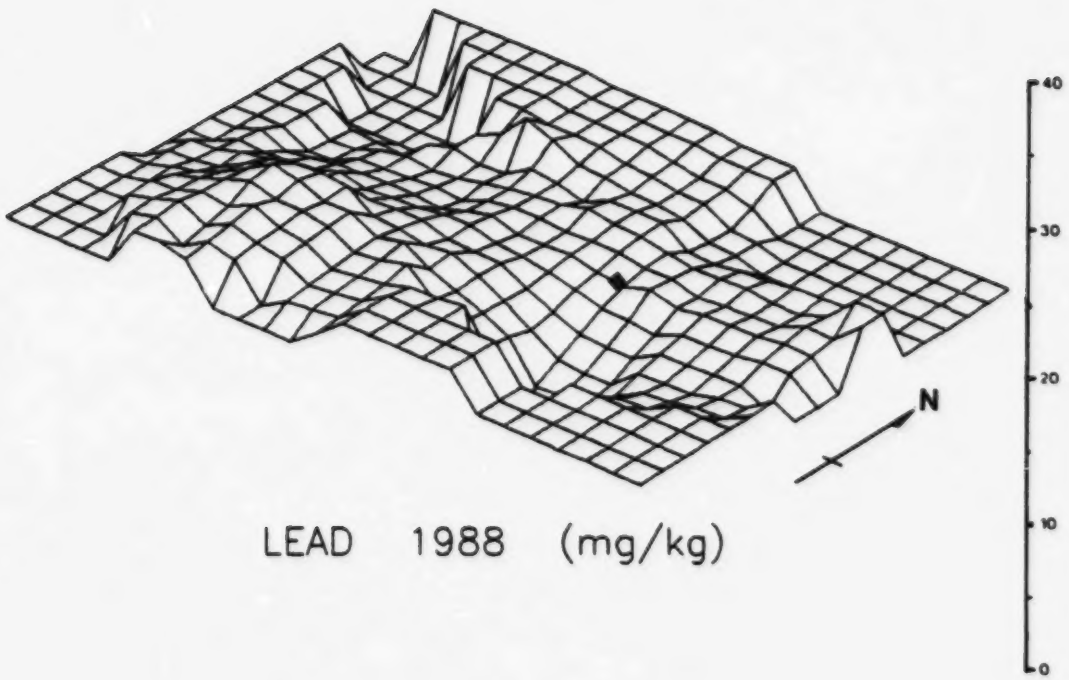


FIGURE 22: Three dimensional representation of 1988 lead data in the dumpsite sampling area. Dumpsite centre (◆).

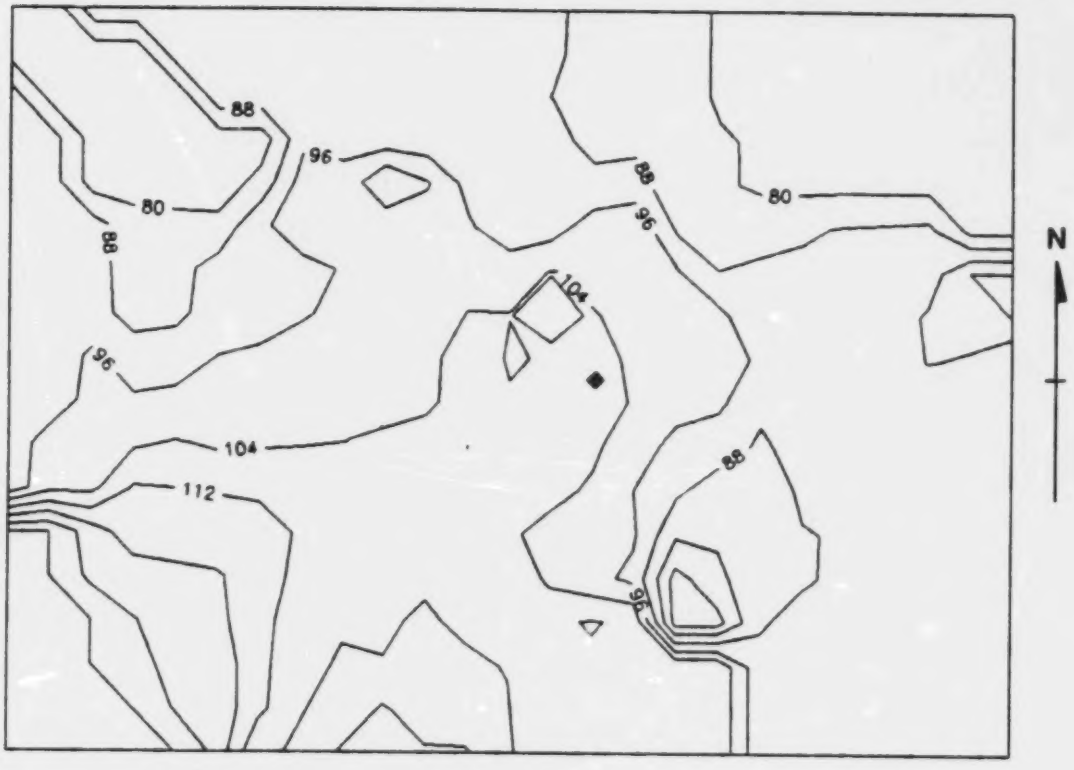
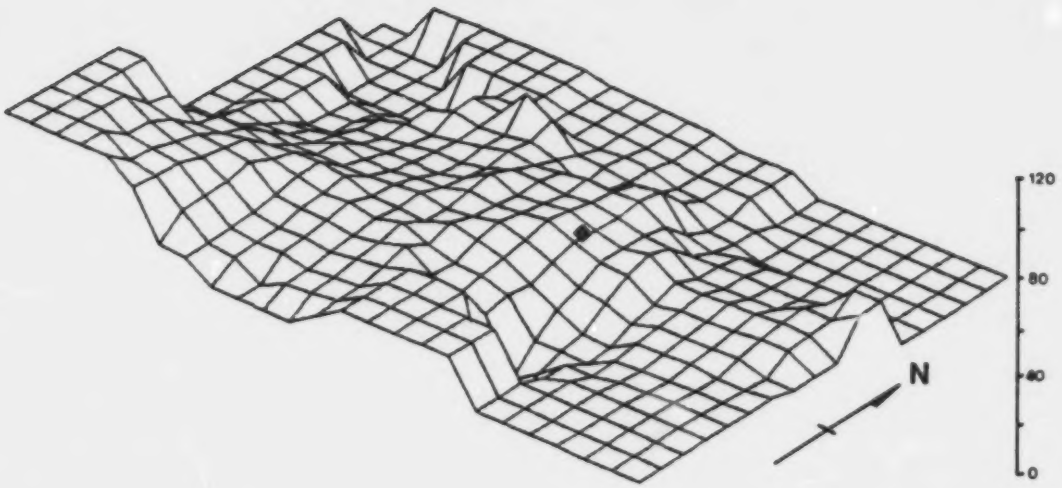


FIGURE 23: Topographical representation of 1988 zinc data in the dumpsite sampling area. Dumpsite centre (◆).



ZINC 1988 (mg/kg)

FIGURE 24: Three dimensional representation of 1988 zinc data in the dumpsite sampling area. Dumpsite centre (◆).

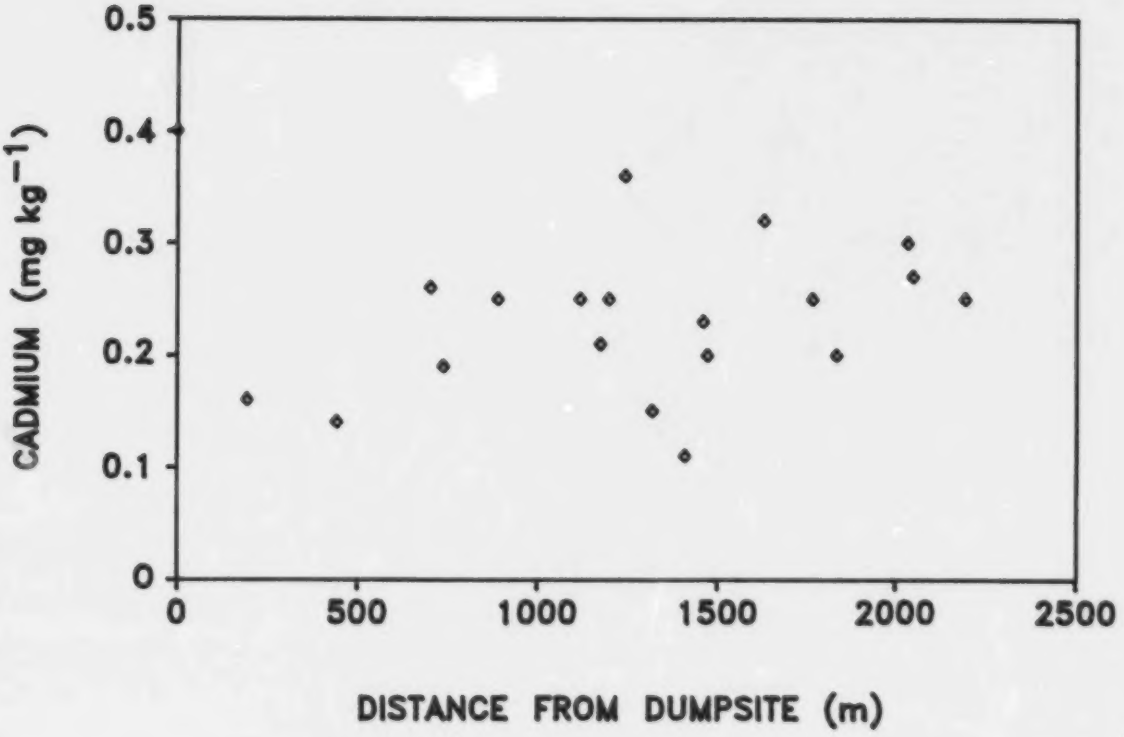


FIGURE 25: Relationship between cadmium concentration and vector distance from the dumpsite.

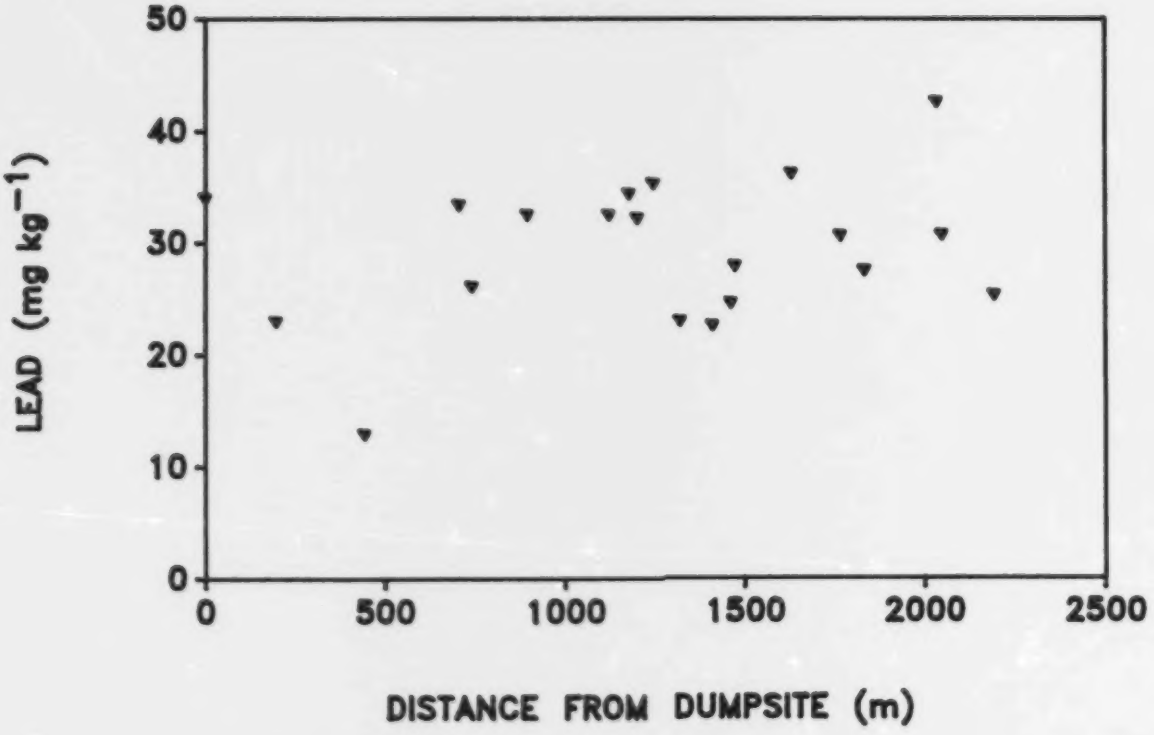


FIGURE 26: Relationship between lead concentration and vector distance from the dumpsite centre.

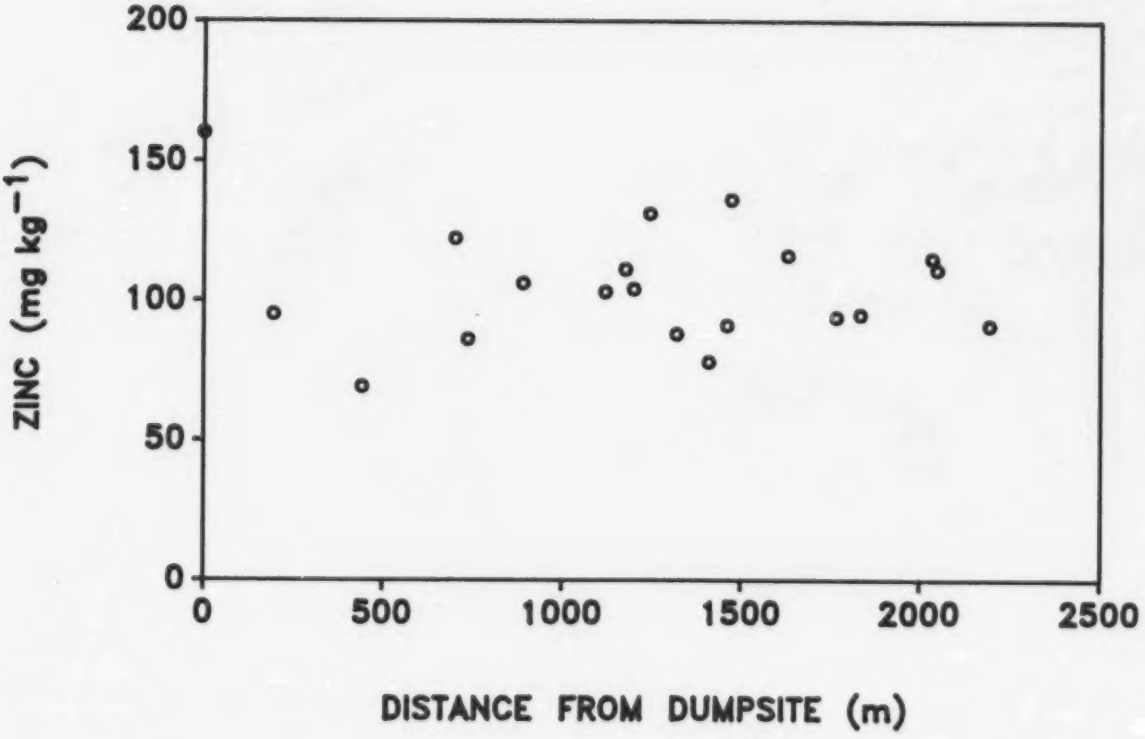


FIGURE 27: Relationship between zinc concentration and vector distance from the dumpsite centre.

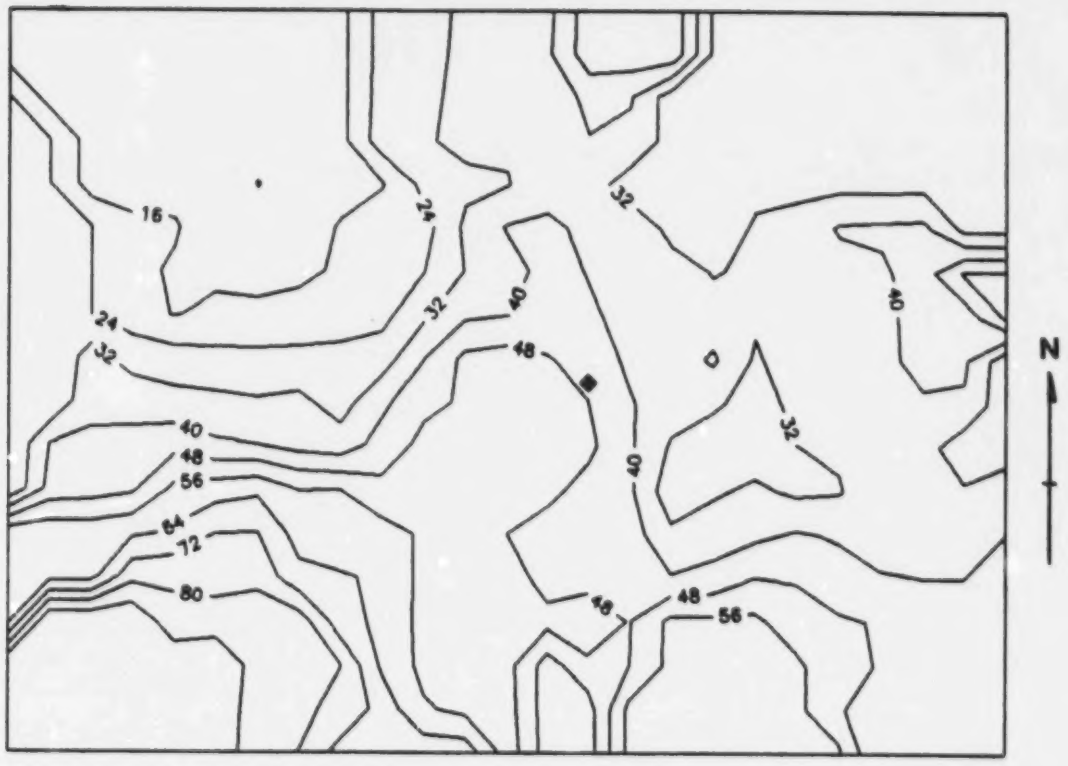
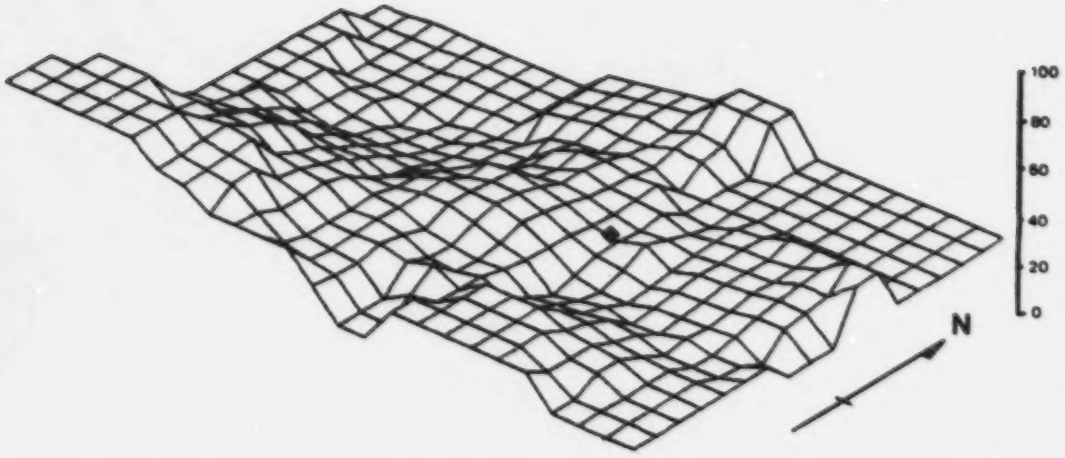
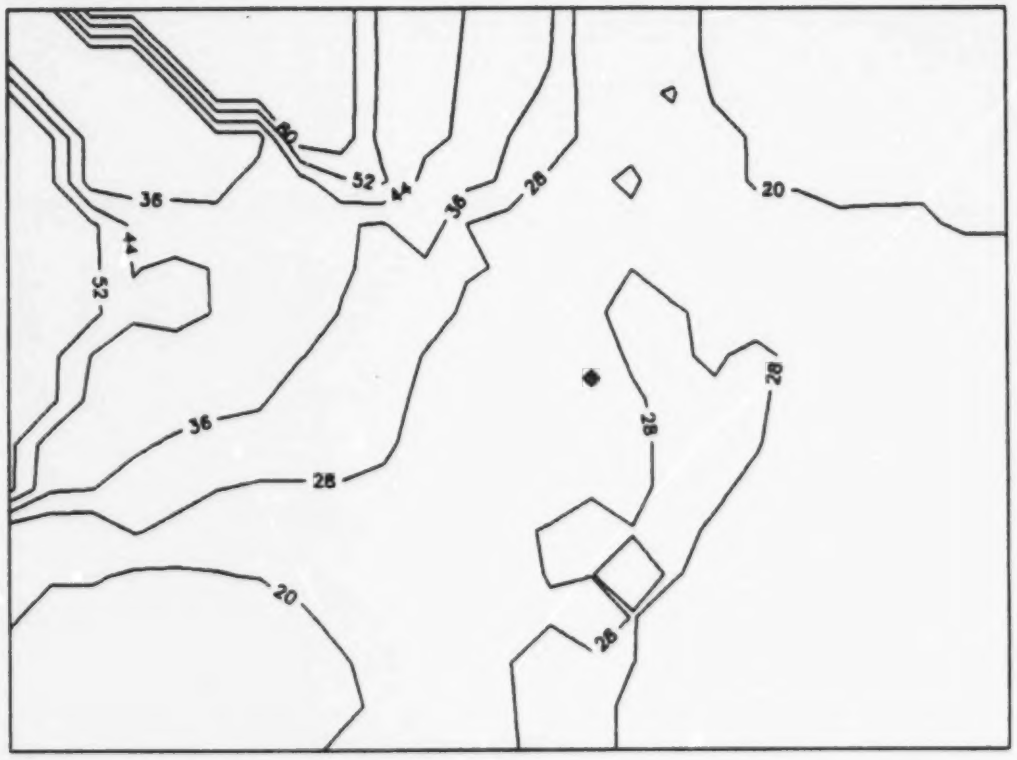


FIGURE 28: Topographical representation of 1988 total inorganic carbon data in the dumpsite sampling area. Dumpsite centre (◆).



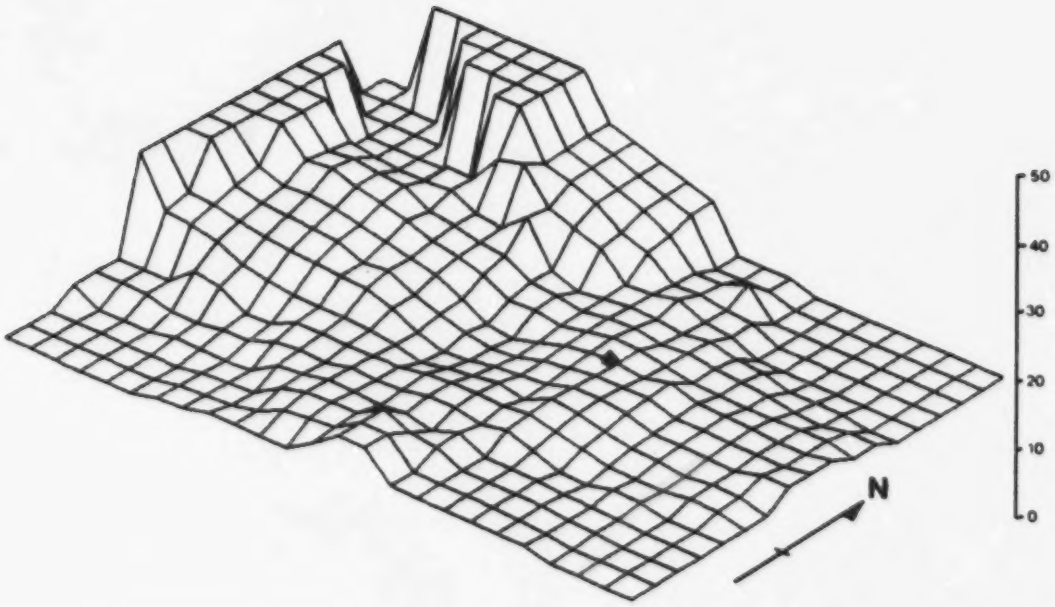
TOTAL INORGANIC CARBON (g/kg)

FIGURE 29: Three dimensional representation of 1988 total inorganic carbon data in the dumpsite sampling area. Dumpsite centre (◆).



TOTAL ORGANIC CARBON (g/kg)

FIGURE 30: Topographical representation of 1988 total organic carbon data in dumpsite sampling area. Dumpsite centre (◆).



TOTAL ORGANIC CARBON (g/kg)

FIGURE 31: Three dimensional representation of 1988 total organic carbon data in the dumpsite sampling area. Dumpsite centre (◆).

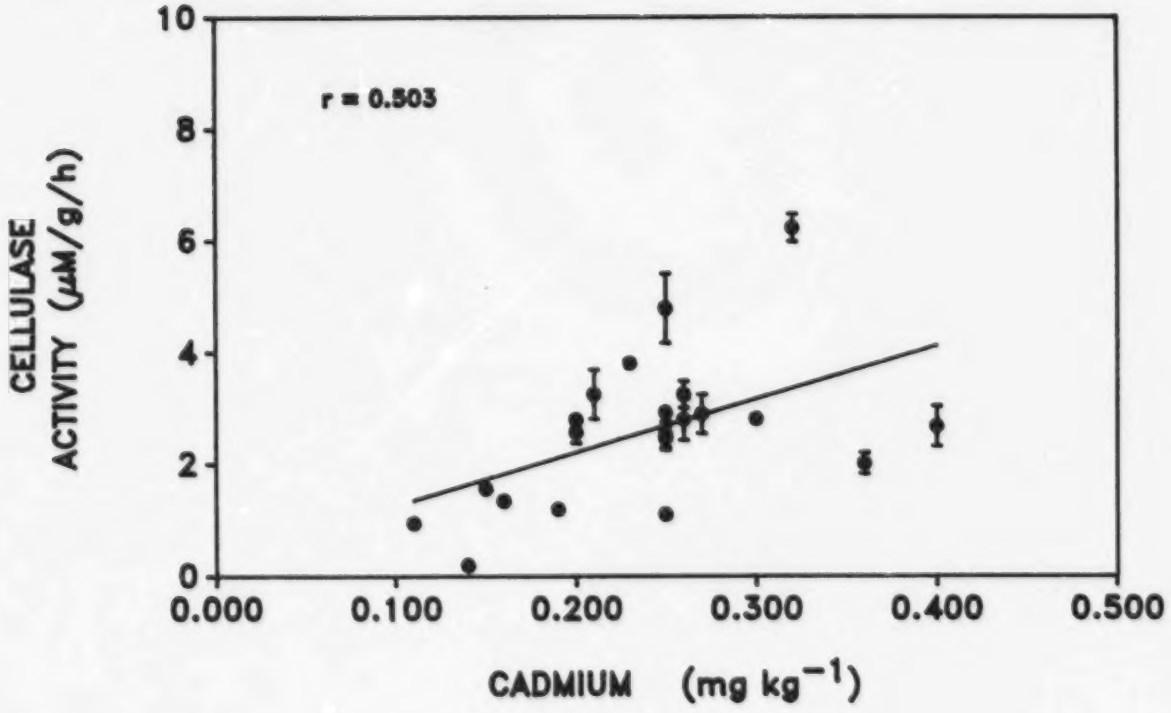


FIGURE 32: Relationship between cellulase activity and sediment cadmium concentration.

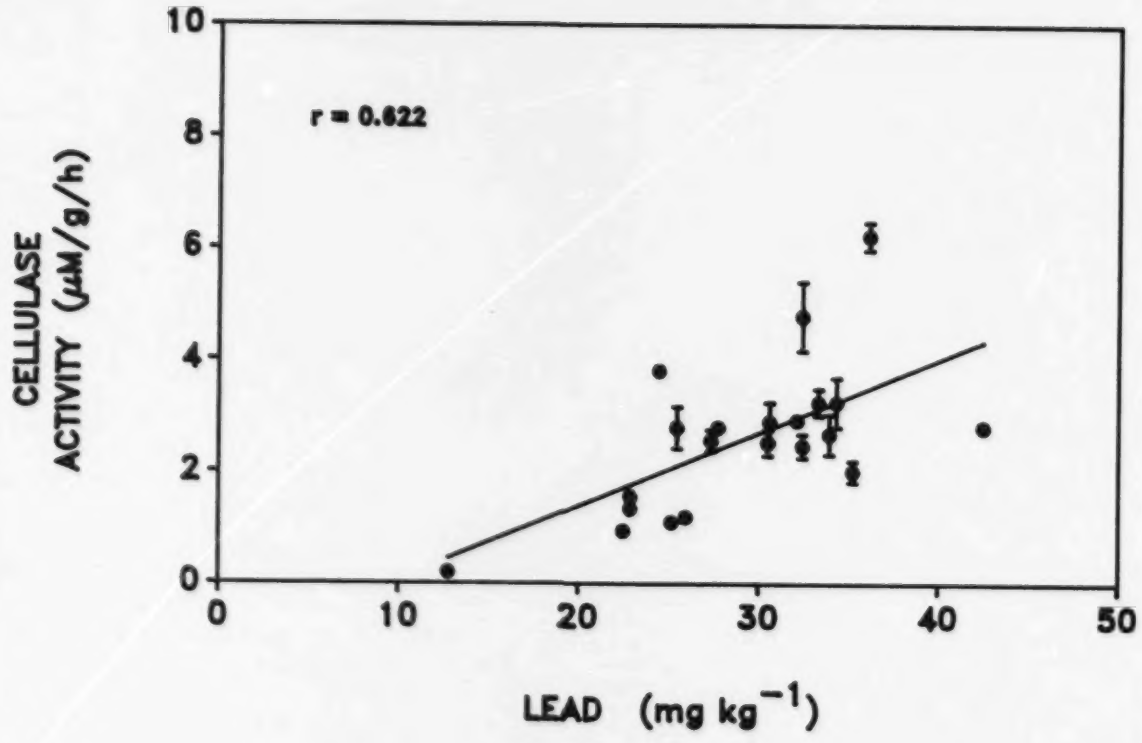


FIGURE 33: Relationship between cellulase activity and sediment lead concentration.

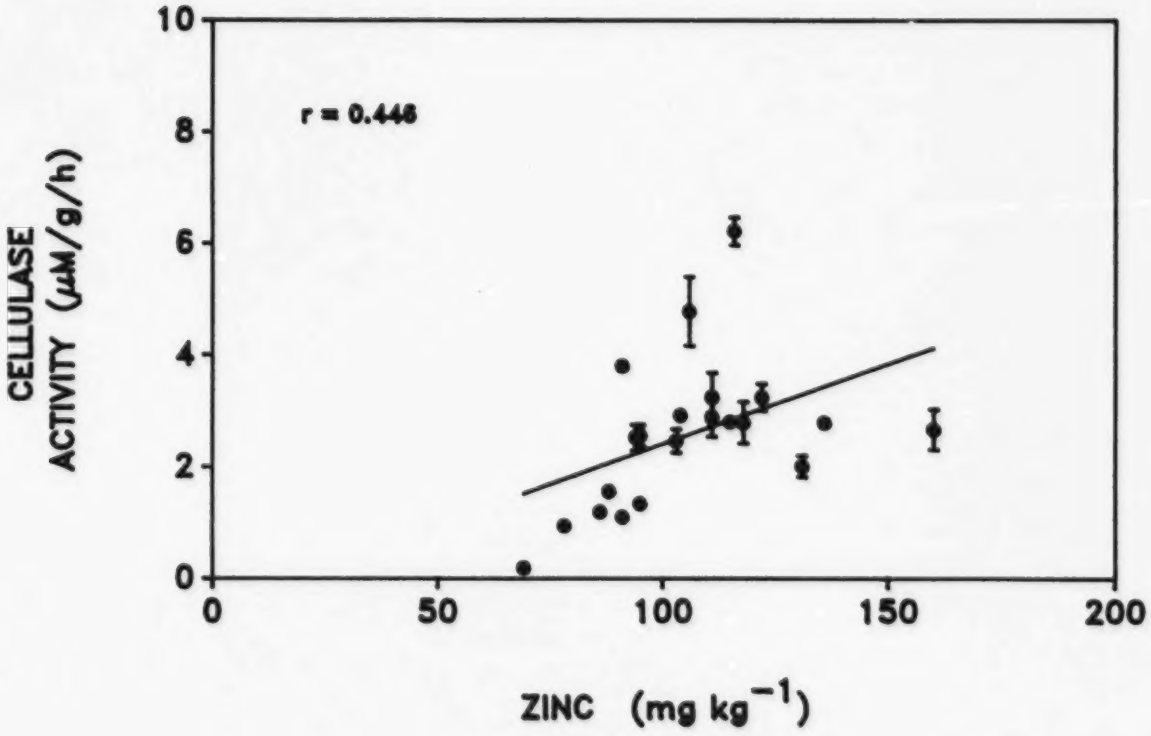


FIGURE 34: Relationship between cellulase activity and sediment zinc concentration

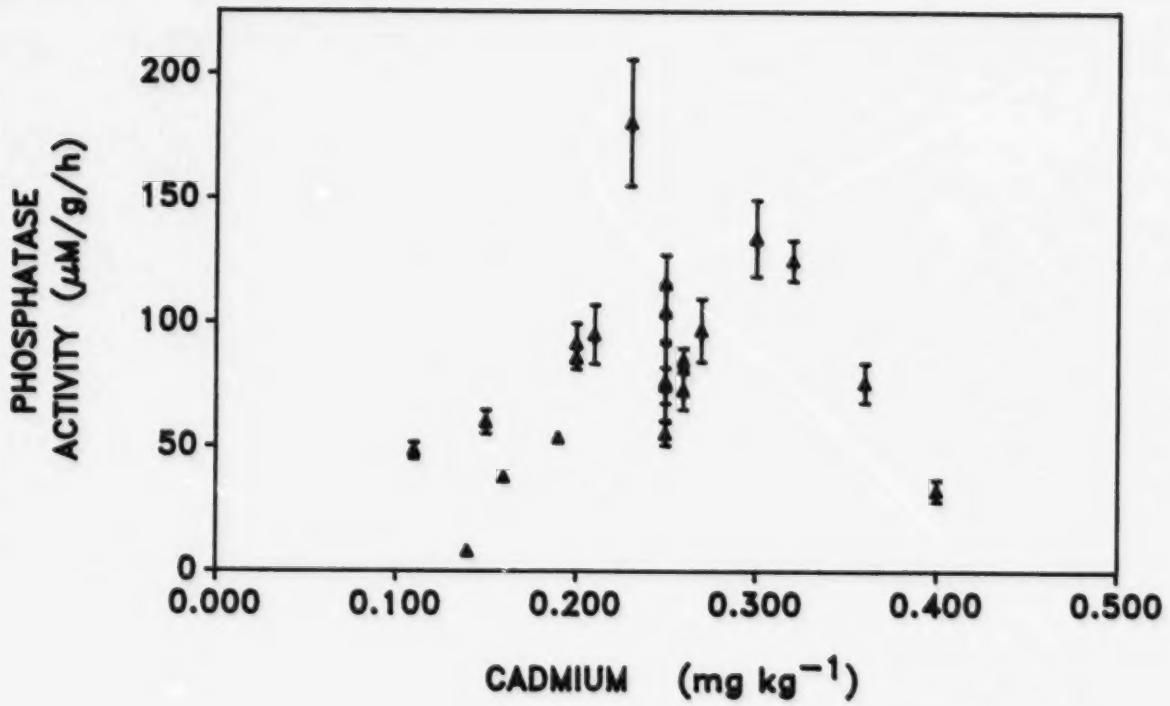


FIGURE 35: Relationship between phosphatase activity and sediment cadmium concentration.

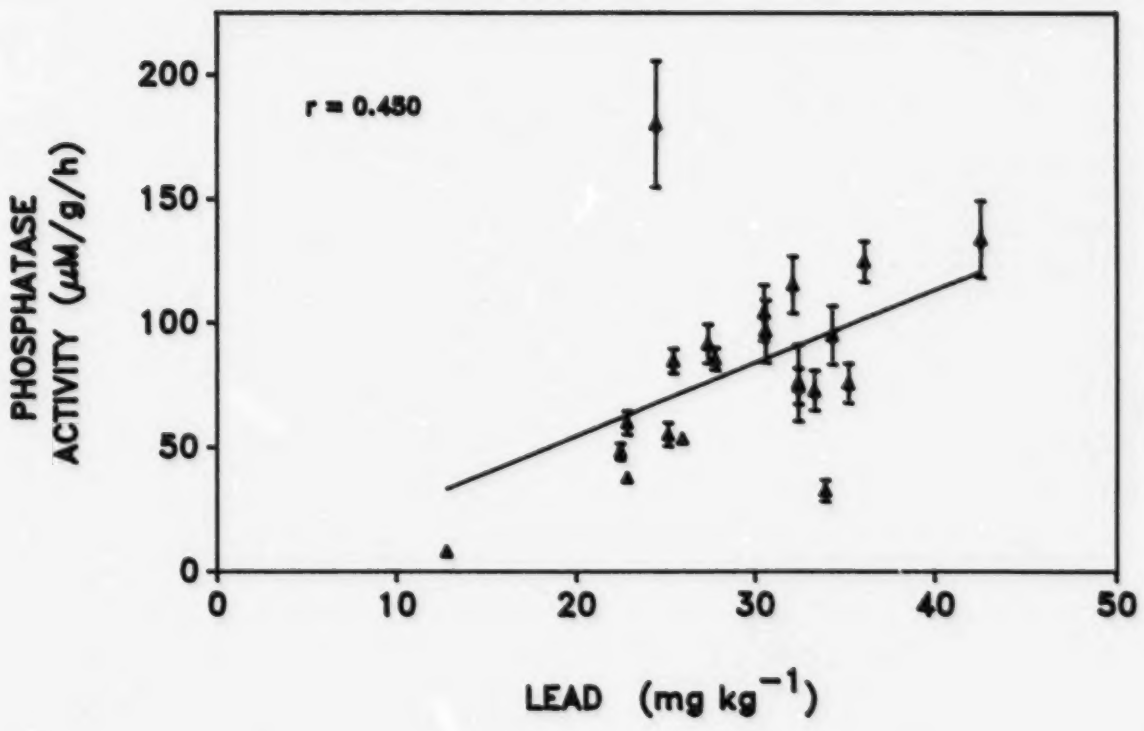


FIGURE 36: Relationship between phosphatase activity and sediment lead concentration.

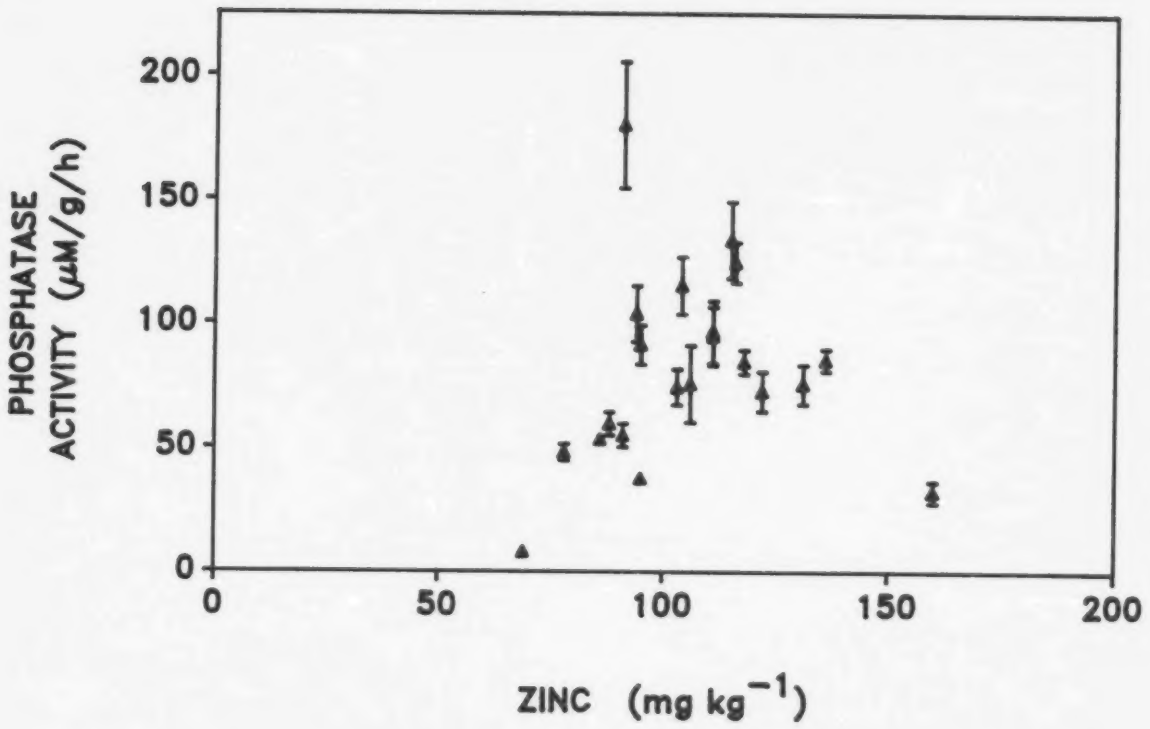


FIGURE 37: Relationship between phosphatase activity and sediment zinc concentration.

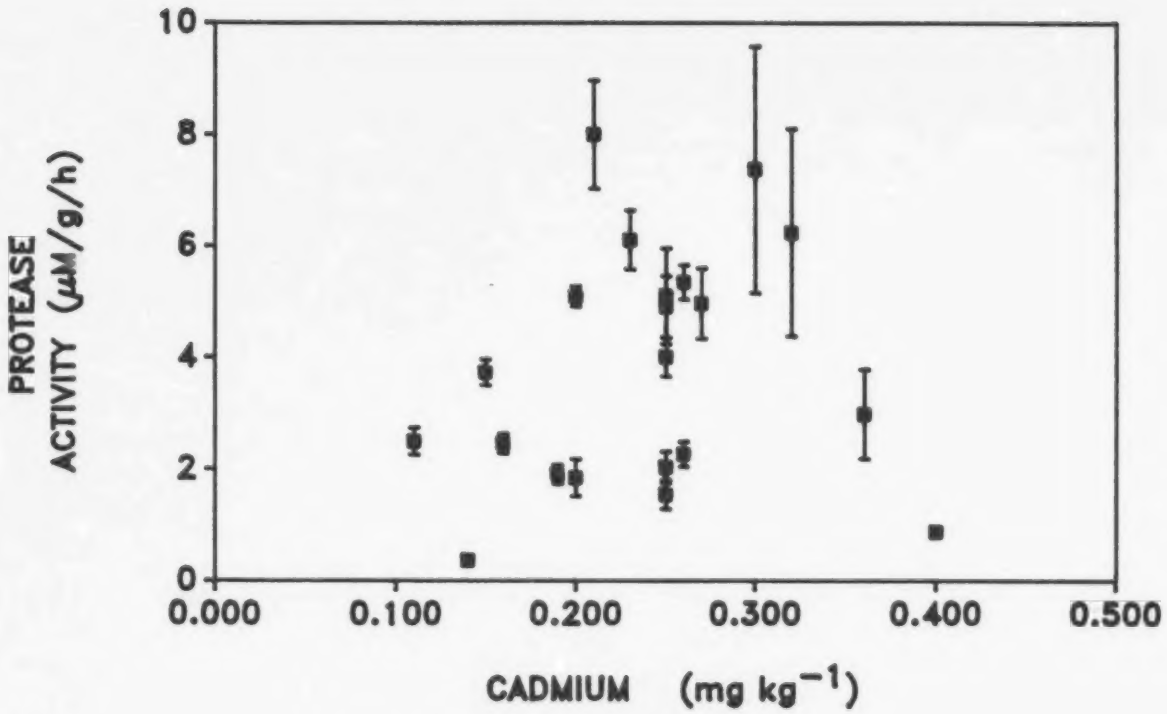


FIGURE 38: Relationship between protease activity and sediment cadmium concentration.

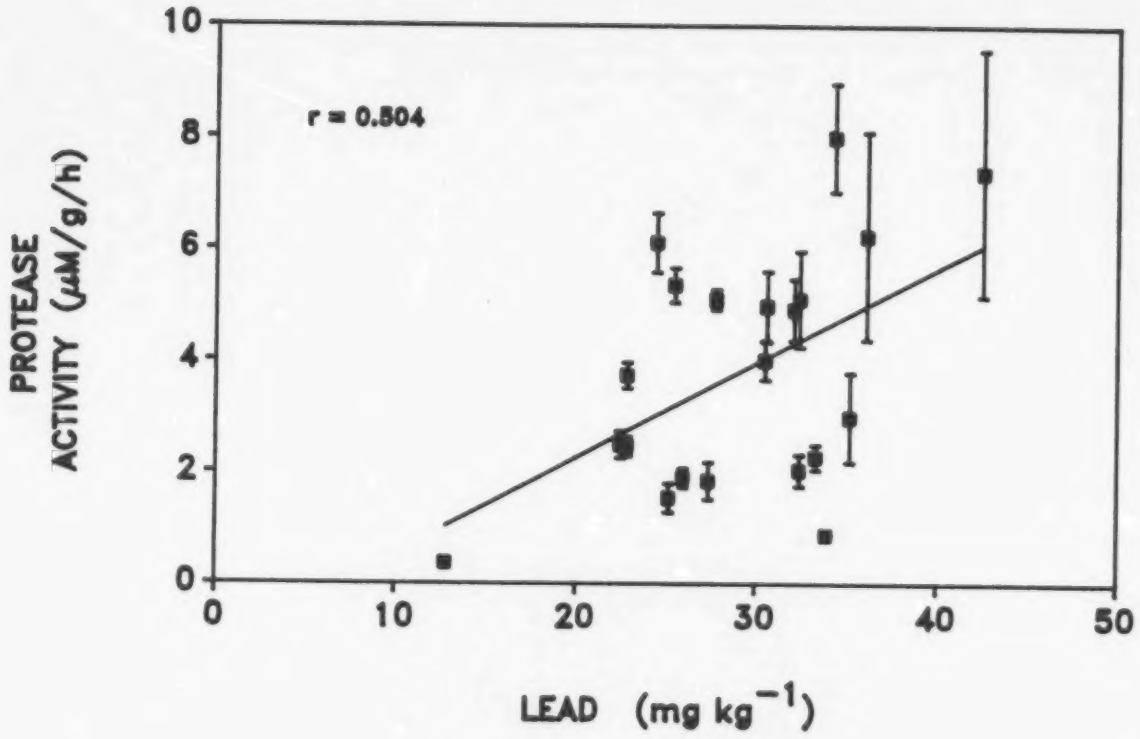


FIGURE 39: Relationship between protease activity and sediment lead concentration.

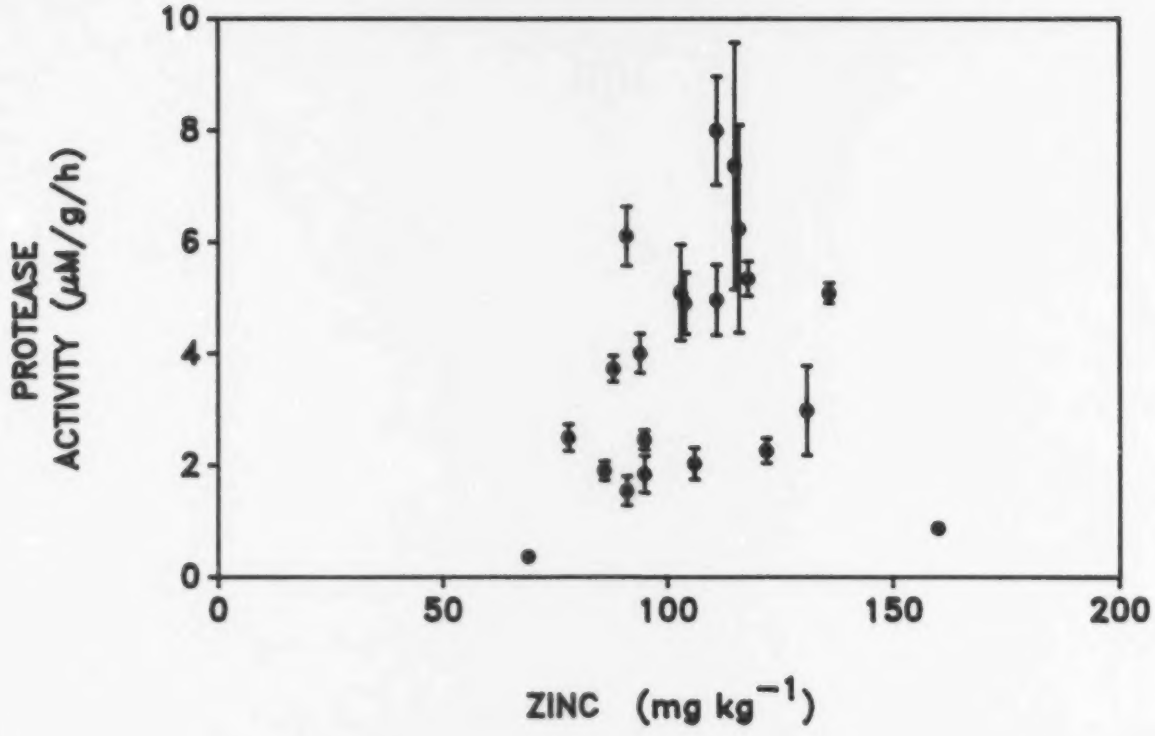


FIGURE 40: Relationship between protease activity and sediment zinc concentration.

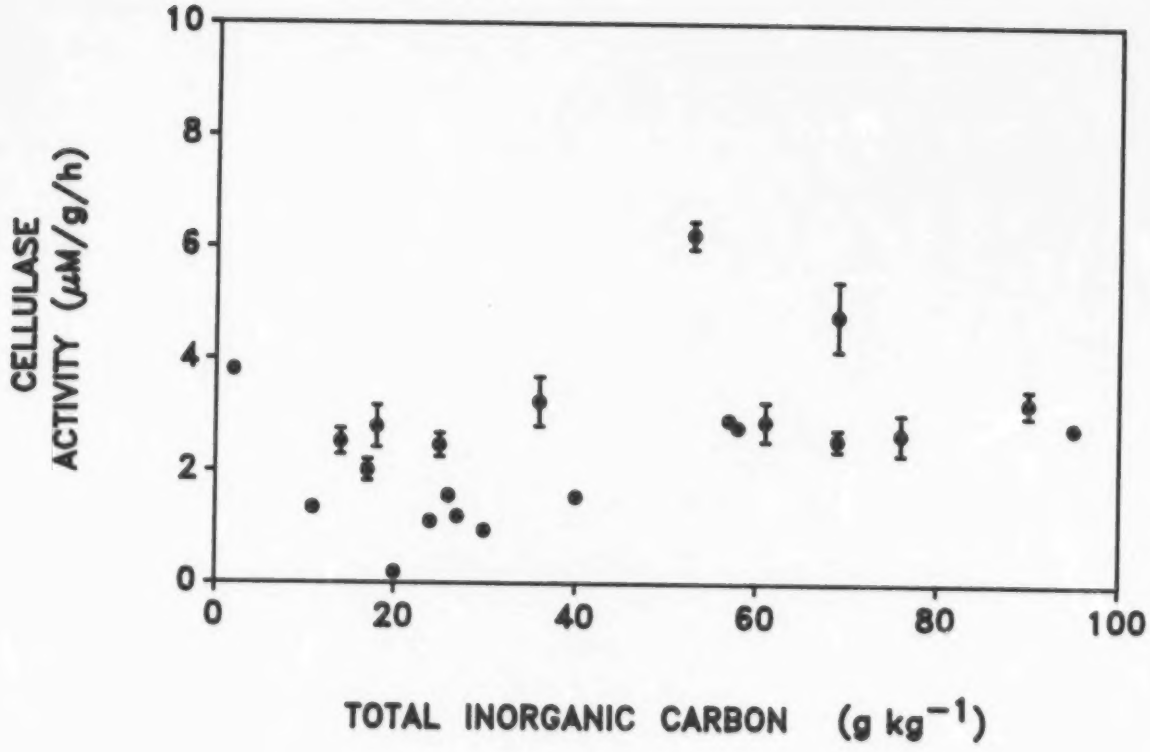


FIGURE 41: Relationship between cellulase activity and total inorganic carbon concentration.

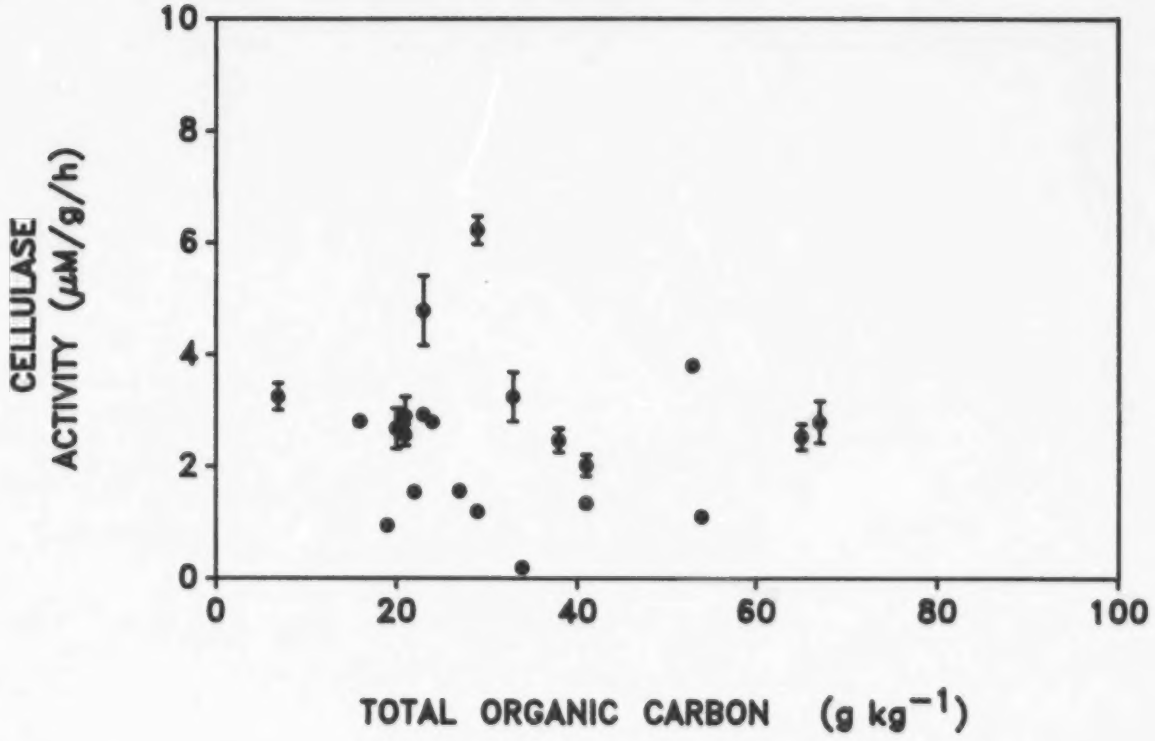


FIGURE 42: Relationship between cellulase activity and total organic carbon concentration.

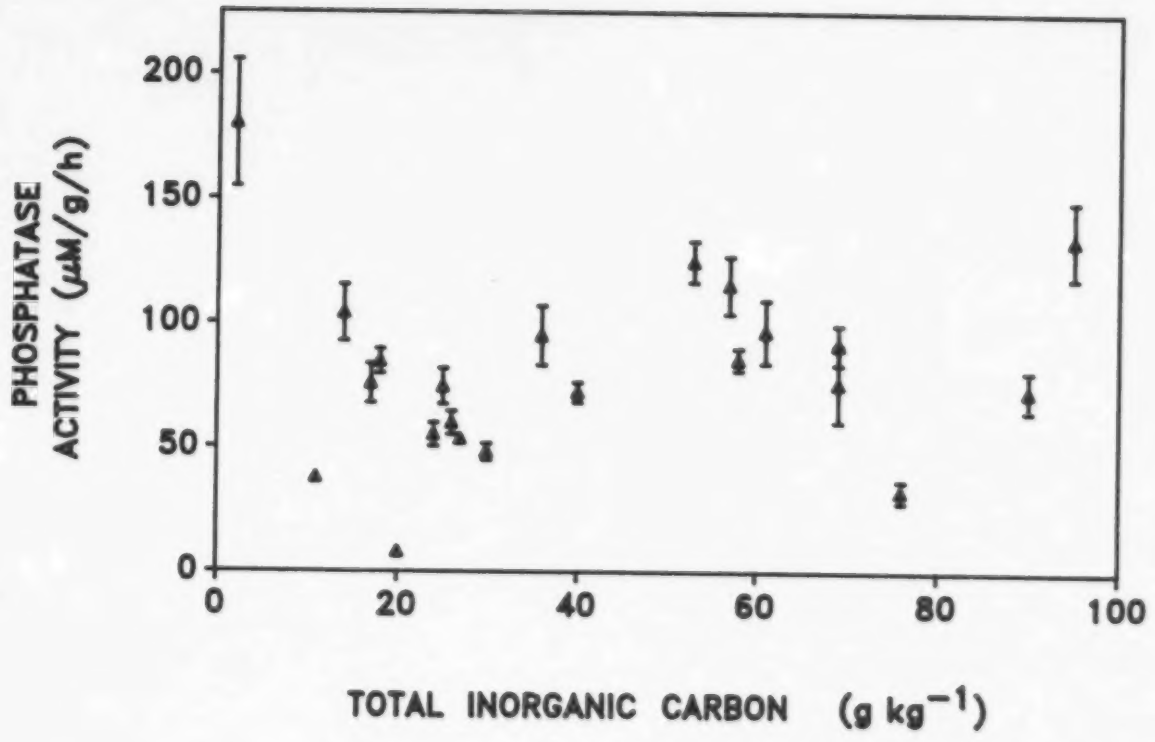


FIGURE 43: Relationship between phosphatase activity and total inorganic carbon concentration.

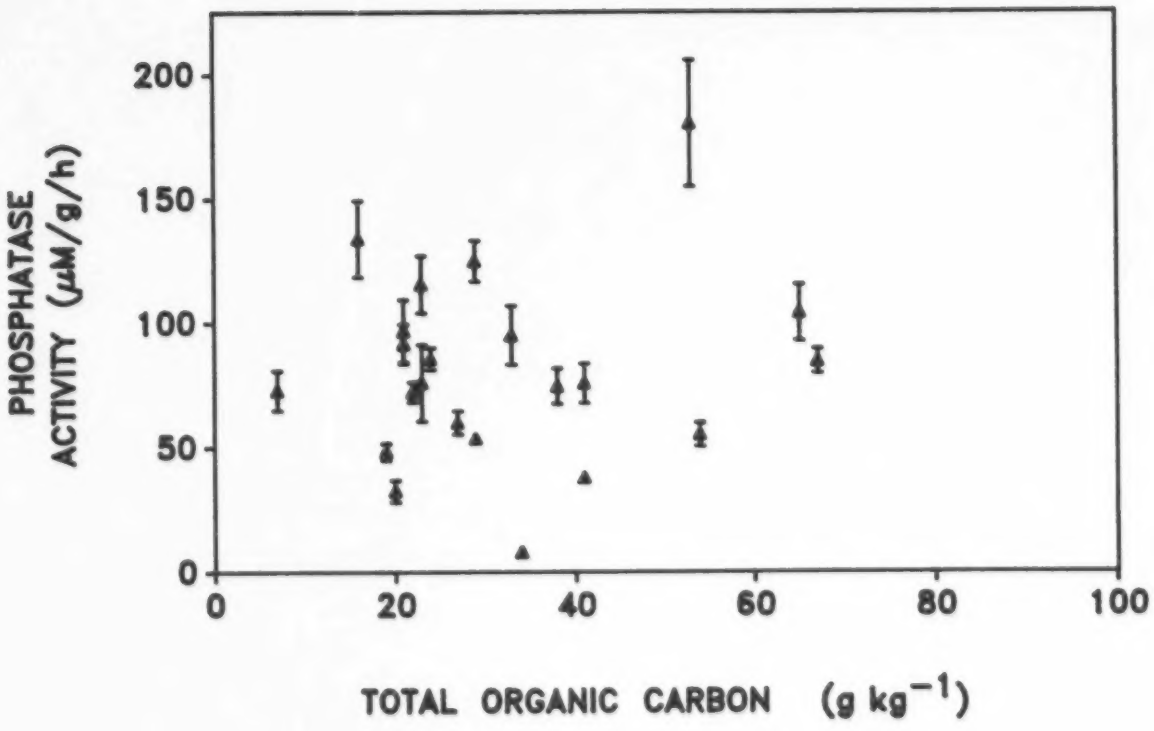


FIGURE 44: Relationship between phosphatase activity and total organic carbon concentration.

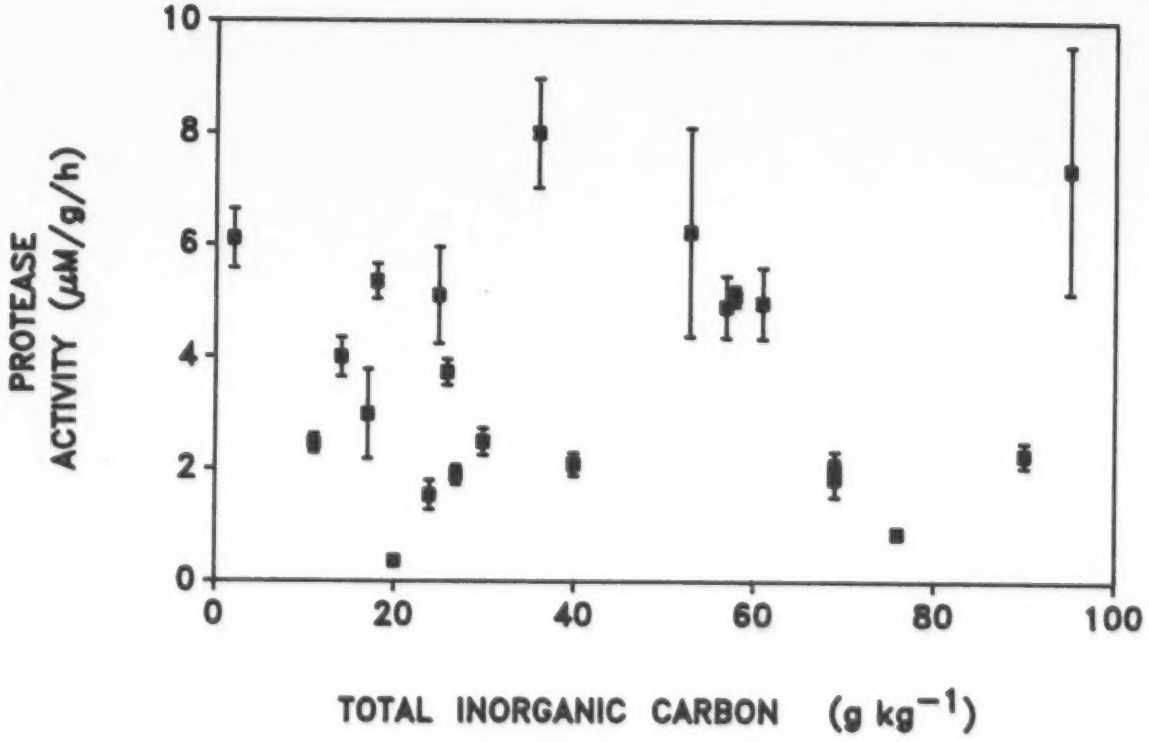


FIGURE 45: Relationship between protease activity and total inorganic carbon concentration.

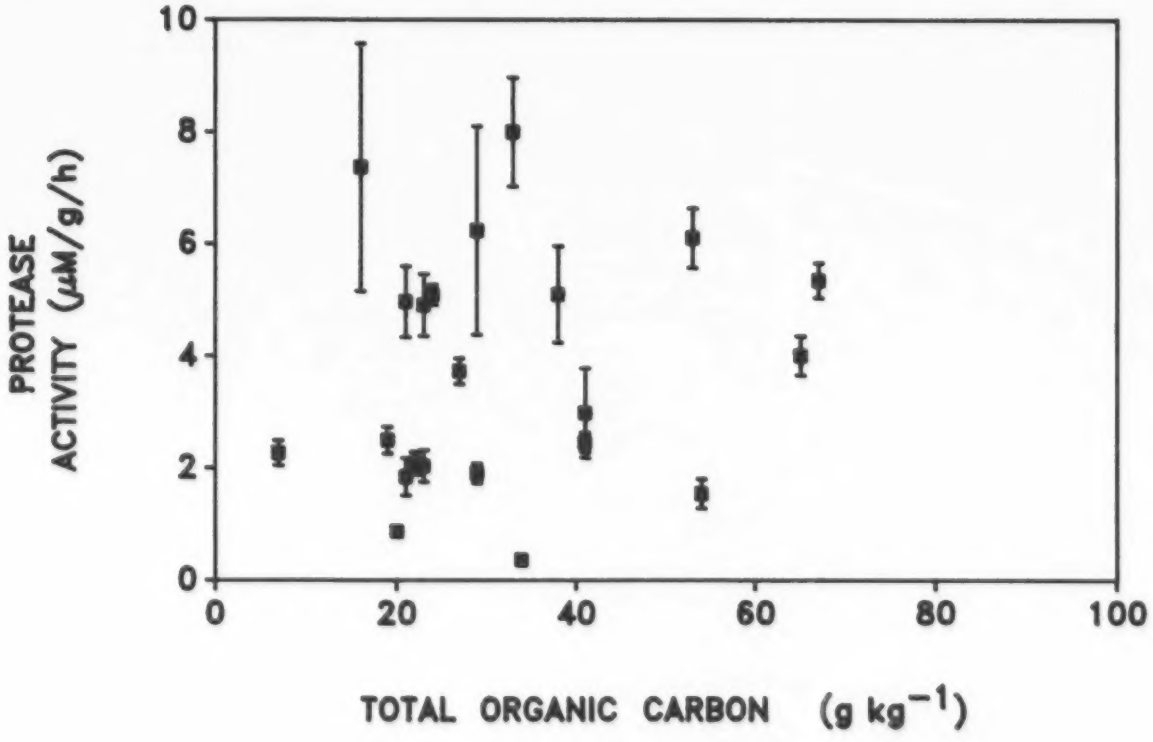


FIGURE 46: Relationship between protease activity and total organic carbon concentration.

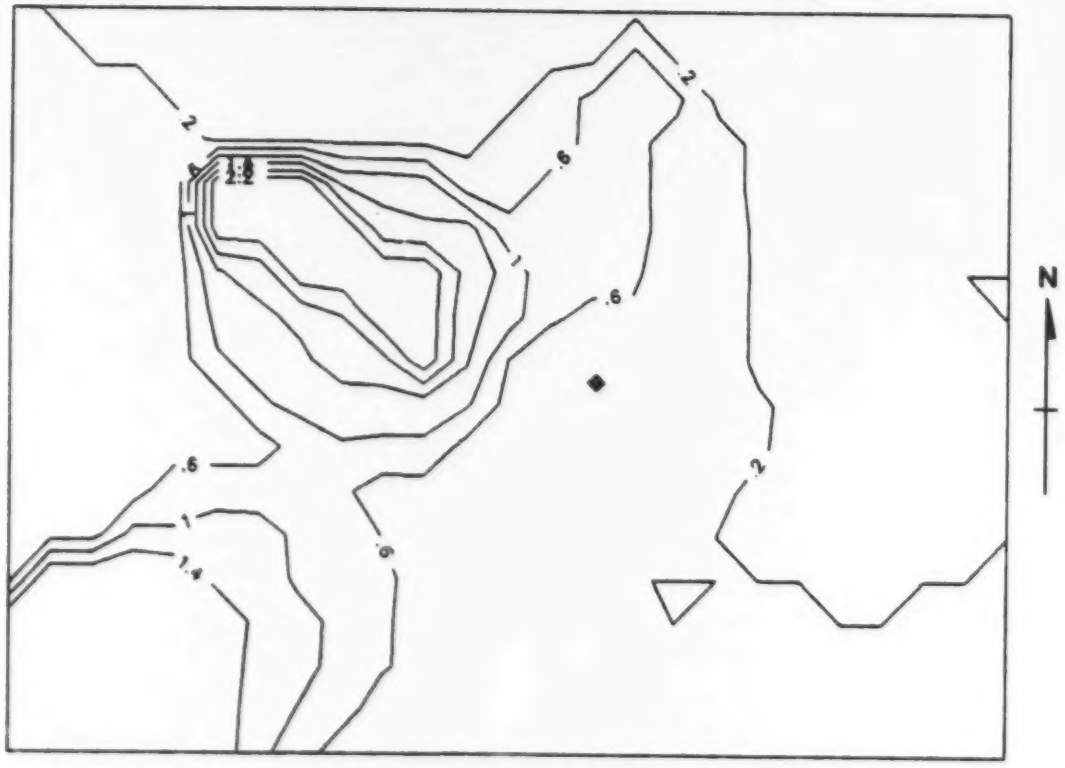
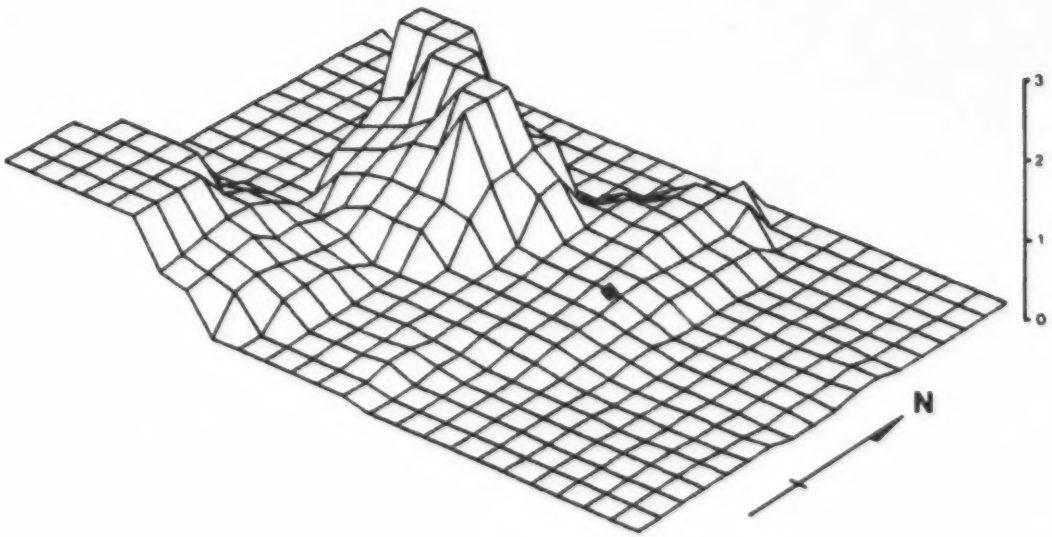


FIGURE 47: Topographical representation of 1981 cadmium data in the dumpsite sampling area. Dumpsite centre (◆).



CADMIUM 1981 (mg/kg)

FIGURE 48: Three dimensional representation of 1981 cadmium data in dumpsite sampling area. Dumpsite centre (◆).

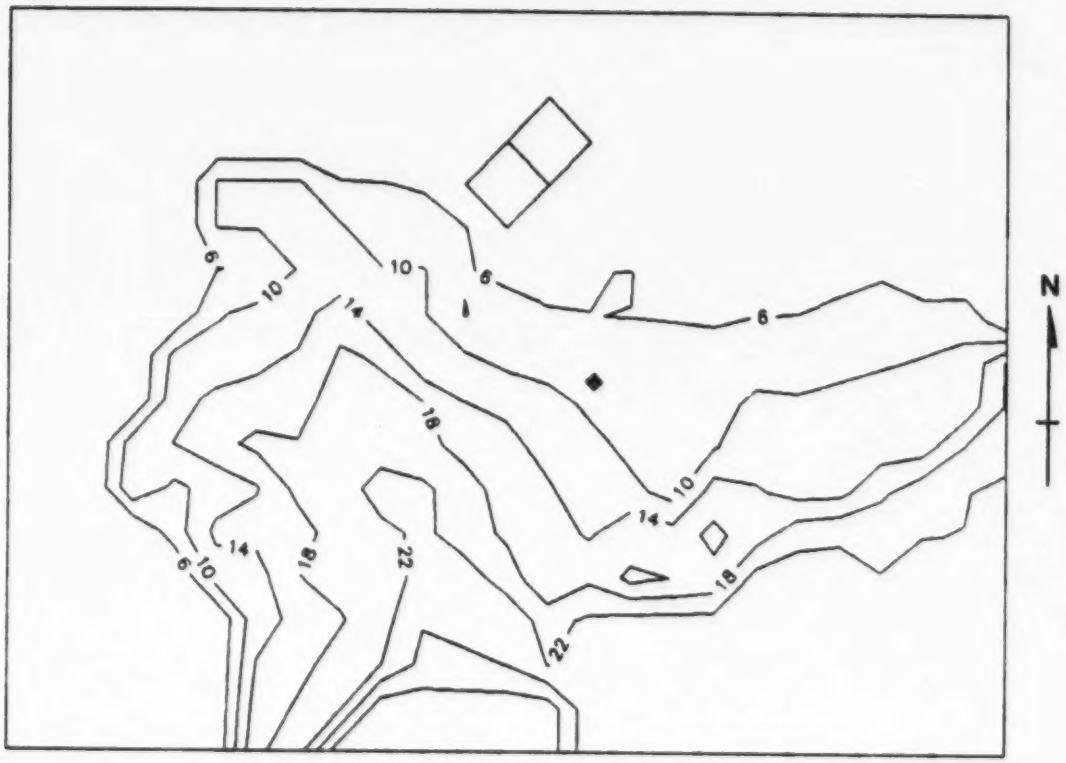


FIGURE 49: Topographical representation of 1981 lead data in the dumpsite sampling area. Dumpsite centre (◆).

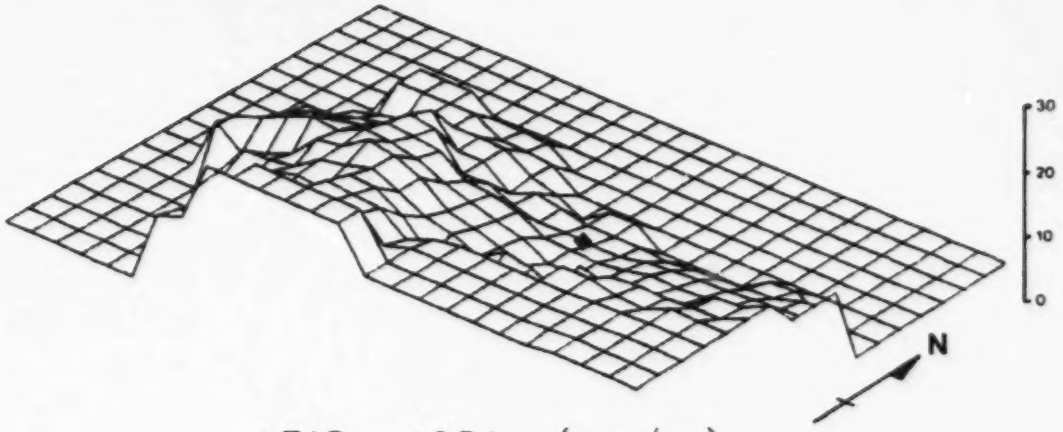
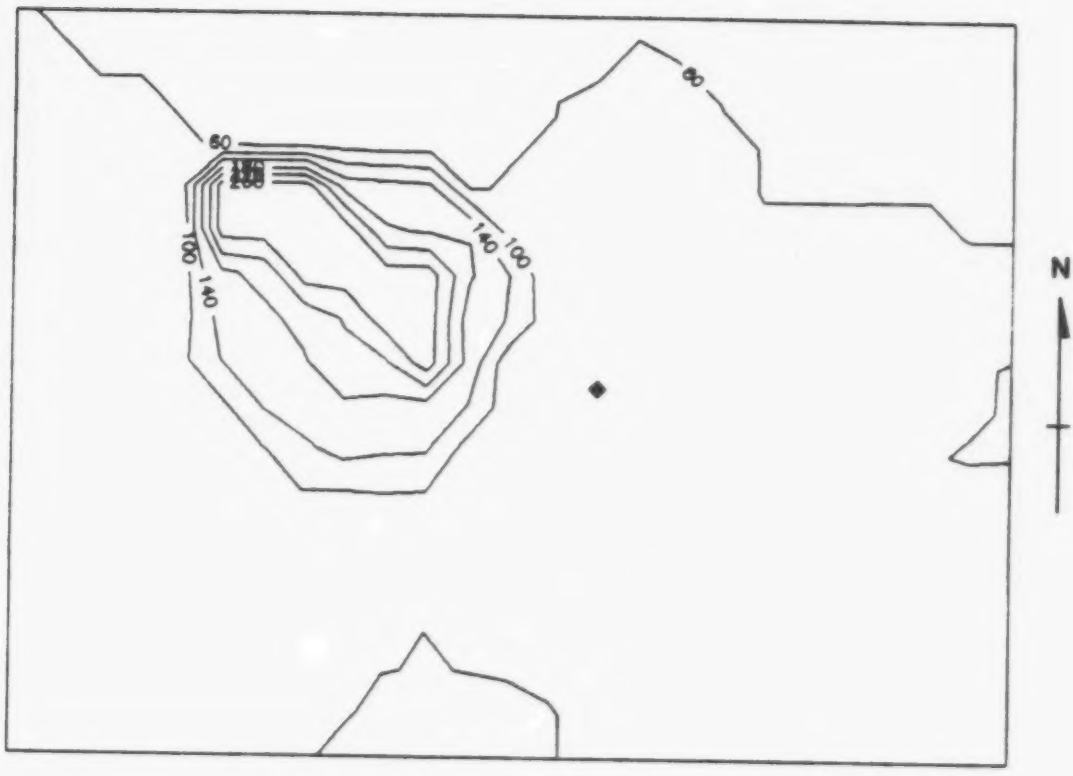
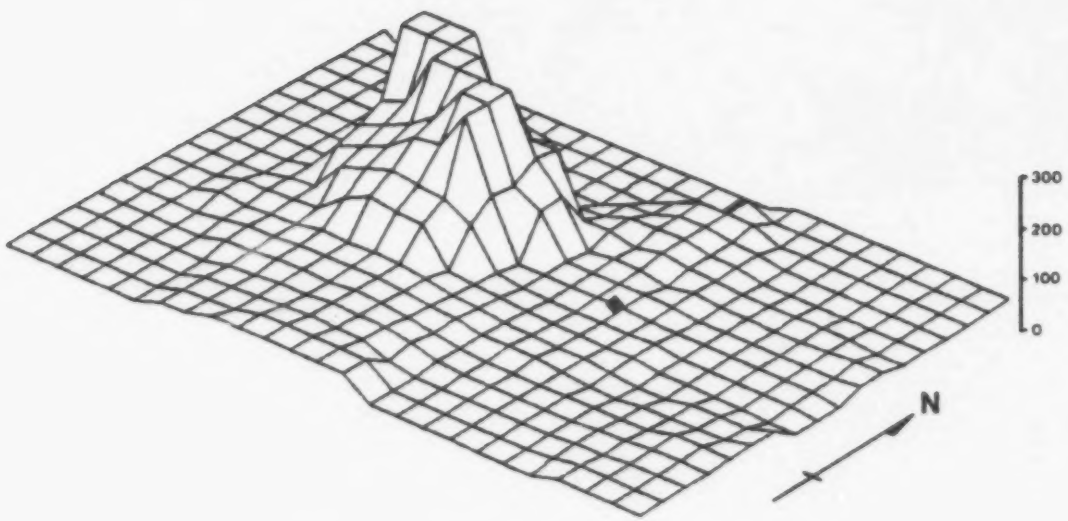


FIGURE 50: Three dimensional representation of 1981 lead data in the dumpsite sampling area. Dumpsite centre (◆).



ZINC 1981 (mg/kg)

FIGURE 51: Topographical representation of 1981 zinc data in the dumpsite sampling area. Dumpsite centre (◆).



ZINC 1981 (mg/kg)

FIGURE 52: Three dimensional representation of 1981 zinc data in the dumpsite sampling area. Dumpsite centre (◆).

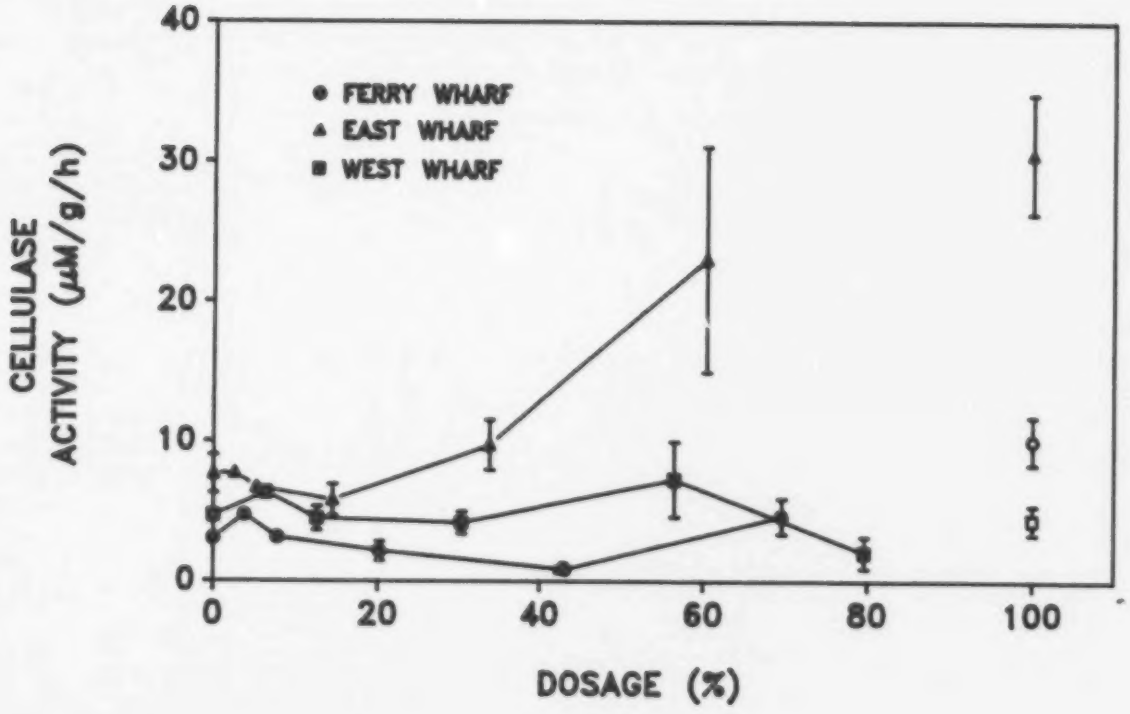


FIGURE 53: Effects of Dalhousie Harbour sediments on cellulase activity in Stn C sediments. Values are mean +/- S.E.

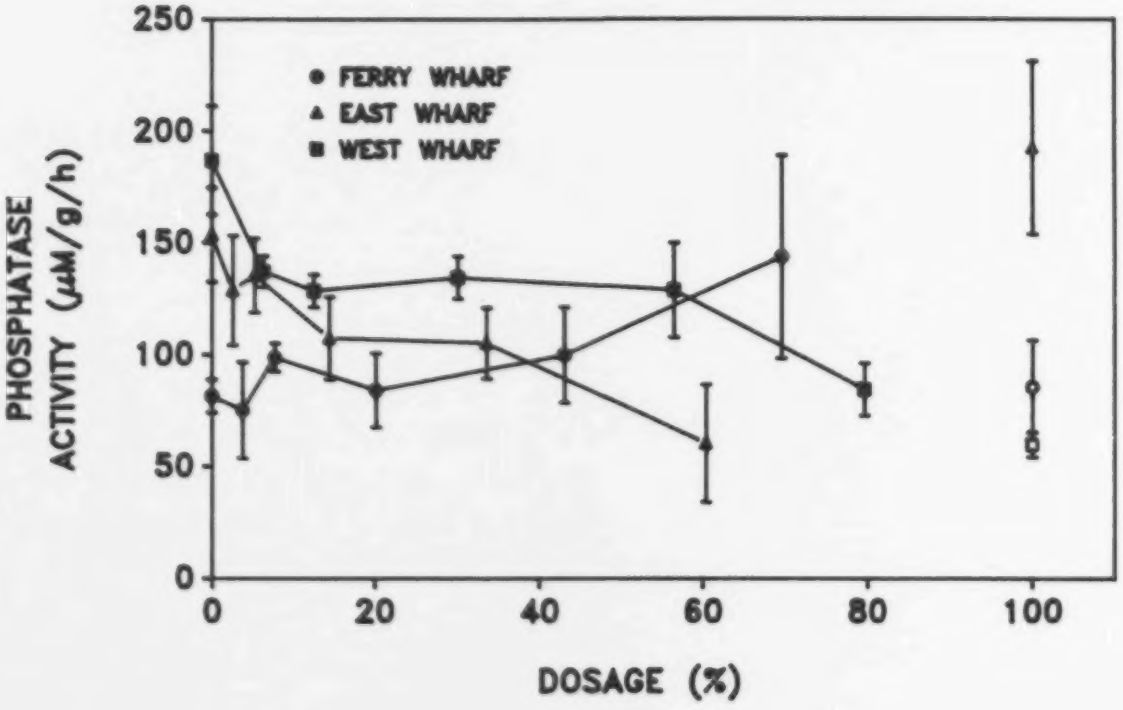


FIGURE 54: Effects of Dalhousie Harbour sediments on phosphatase activity in Stn C sediments. Values are mean +/- S.E.

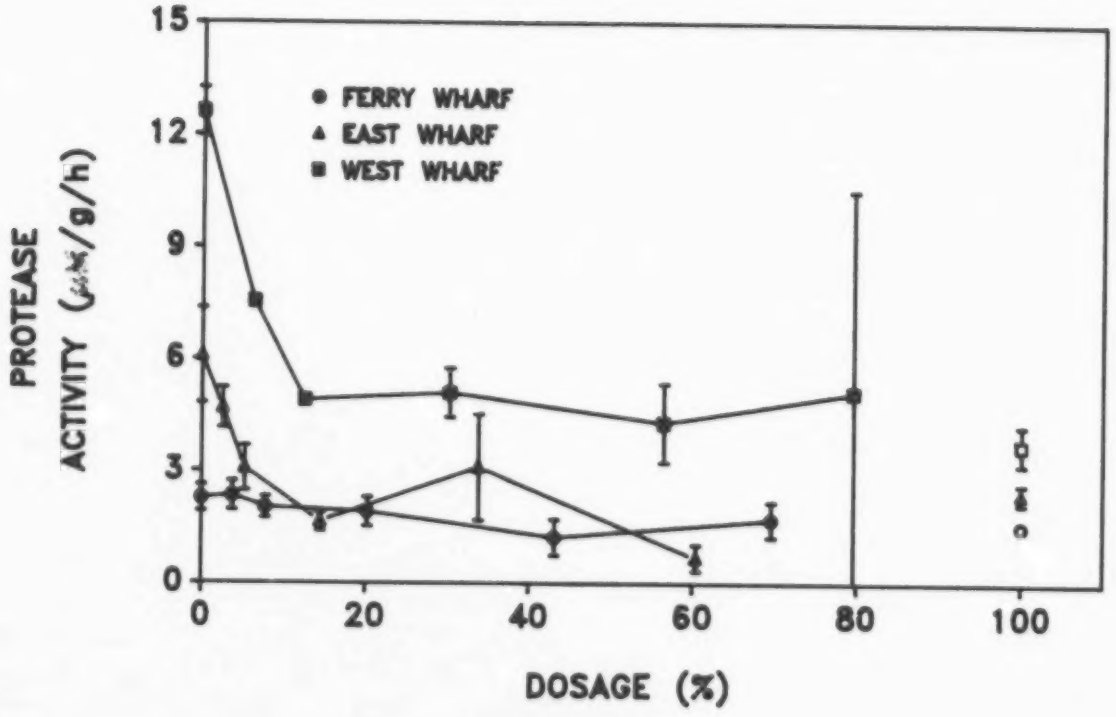


FIGURE 55: Effects of Dalhousie Harbour sediments on protease activity in Stn C sediments. Values are mean +/- S.E.

9.0 APPENDIX

The Microsoft(tm) GW-BASIC program CONVERT.BAS, listed below, was used to convert the total enzyme activity in each bioassay tube to the enzyme activity in the impacted Station C sediment.

```
5 REM  PROGRAM "CONVERT.BAS" IN GW-BASIC
10 CLEAR : CLS
15 DIM CONC(10), ACTIV(100,3), RESULTS(10,3)
16 GOTO 230
20 INPUT "Enter number of toxicant concentrations used (incl. ctrl) ";NCONC : PR
INT : INPUT "Enter number of replicates at each concentration "; NREP
30 PRINT : PRINT : FOR I = 1 TO NCONC : PRINT "CONC. #"; I;: INPUT CONC(I) : PRI
NT : NEXT I
40 CLS
45 PRINT" INPUT ACTIVITY IN EACH TUBE"
50 FOR J = 1 TO (NCONC * NREP) : PRINT : PRINT " TUBE "; J; : INPUT ACTIV(J,1) :
 NEXT J : CLS
55 K = (NCONC * NREP / 2) : K% = K : RES = K - K% : IF RES = 0 THEN 60 ELSE K =
(NCONC * NREP / 2) - .5
60 FOR I = 1 TO K : PRINT "TUBE "; I; "  ";ACTIV(I,1), "TUBE ";(I+K); "  "; ACTI
V(I+K,1) : NEXT I : IF RES < 0 THEN PRINT , , "TUBE "; (NCONC * NREP); "  " ACTI
V(NCONC * NREP,1)
70 PRINT : PRINT : INPUT "ARE ALL THESE VALUES CORRECT (Y/N) "; ANS$ : IF ANS$ =
 "Y" THEN 100
80 INPUT "WHICH VALUE IS INCORRECT "; NUM : INPUT "ENTER CORECT VALUE "; ACTIV(N
UM,1) : GOTO 60
90 REM
95 TOX = 0 : ACTOX = 0
100 FOR J = 1 TO NREP : TOX = TOX + ACTIV(J + (NREP * (NCONC - 1)),1) : NEXT J :
 TOX = TOX / NREP
105 FOR P = 1 TO NREP : ACTIV(P,2) = ACTIV(P,1) : ACTIV(P,3) = ACTIV(P,1) : NEXT
 P : FOR R = (NCONC * NREP - NREP + 1) TO (NCONC * NREP) : ACTIV(R,3) = ACTIV(R,
1) : NEXT R
110 FOR L = 2 TO NCONC-1 : ACTOX = TOX * CONC(L) / 100 : FOR M = (L * NREP - 2)
TO (L * NREP) : ACTIV(M,2) = ACTIV(M,1)-ACTOX : ACTIV(M,3) = ACTIV(M,2) * 100 /
(100 - CONC(L)) : NEXT M : NEXT L
115 FOR P = 1 TO NCONC : HOLD = 0 : EX2 = 0
120 FOR Q = (P * NREP -NREP + 1) TO (P * NREP) : HOLD = HOLD + ACTIV(Q,3) : EX2
= EX2 + (ACTIV(Q,3)^2) : NEXT Q
130 RESULTS(P,1) = HOLD / NREP : RESULTS (P,2) = SQR((EX2 - (HOLD^2 / NREP)) / (
NREP - 1)) : RESULTS(P,3) = RESULTS(P,2) / (SQR(NREP))
140 NEXT P
145 NT = NCONC * NREP
150 OPEN "O", #1, "CONVERT.DAT"
160 PRINT #1, NT, NCONC
170 FOR H = 1 TO NT : PRINT #1, ACTIV(H,1), ACTIV(H,2), ACTIV(H,3) : NEXT H
```

```
180 FOR I = 1 TO NCONC : PRINT #1, CONC(I) : PRINT #1, RESULTS(I,1), RESULTS(I,2
), RESULTS(I,3) : NEXT I
190 CLOSE #1
200 CLS
210 INPUT "ENTER NAME FOR DATA FILE ";NOM$
220 NAME "CONVERT.DAT" AS NOM$
230 GOSUB 1000
240 CLS
250 END
1000 CLS
1005 PRINT TAB(20) "ENZYME DATA CONVERSION"
1010 FOR X = 1 TO 10 : PRINT : NEXT X : PRINT TAB(20) "C - Convert activity data
" : PRINT : PRINT TAB(20) "P - Print converted data" : PRINT : PRINT TAB(20) "Q
- Quit" : FOR X = 1 TO 5 : PRINT : NEXT X
1020 PRINT TAB(20) "Enter Choice ";: CH$ = INPUT$(1)
1030 IF CH$ = "C" THEN CLS : GOTO 20
1040 IF CH$ = "P" THEN GOSUB 2000
1050 IF CH$ = "Q" THEN 1060 ELSE GOTO 1000
1060 RETURN
2000 CLS : INPUT "Name of data file "; FILE$
2010 OPEN "I", #1, FILE$
2020 INPUT #1, NT, NCONC
2030 LPRINT TAB(30) " DATA FILE "; FILE$ : LPRINT : LPRINT
2040 LPRINT TAB(15) "TUBE ACT.        ACT. Stn C          ACT. C / g" : LPRINT
2050 FOR J = 1 TO NT : LPRINT "     TUBE #"; J; "    "; : FOR I = 1 TO 3 : INPUT #
1, TEMP : LPRINT TEMP; "           "; : NEXT I : LPRINT " " : LPRINT : NEXT J
2060 LPRINT CHR$(12)
2070 LPRINT TAB(30) "DATA FILE "; FILE$ : LPRINT : LPRINT
2080 LPRINT TAB(15) "CONC.          MEAN ACT.       STD. DEV.       STD. ERR." :
LPRINT
2090 FOR U = 1 TO NCONC : LPRINT "                  "; : INPUT #1, CONC : LPRINT C
ONC; "       ";: FOR V = 1 TO 3 : INPUT #1, TEMP : LPRINT TEMP; "       "; : NEXT
V : LPRINT " " : LPRINT : NEXT U
2100 CLOSE #1
2110 LPRINT CHR$(12)
2120 CH$ = " "
2130 RETURN
```